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Microbiology of.

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microorganisms,

Cane Juices,

Beet Juices,

fermentation,

~~Beet Diffusion Juices~~

sugar beets,

microbiology

THE MICROBIOLOGY OF SUGARS, SYRUPS AND MOLASSES

A Reference Book for the Raw Sugar Manufacturer or Refiner and for All Industries Engaged in the Manufacture, Storage or Commercial Utilization of Sugars, Syrups and Molasses

By WILLIAM L. OWEN
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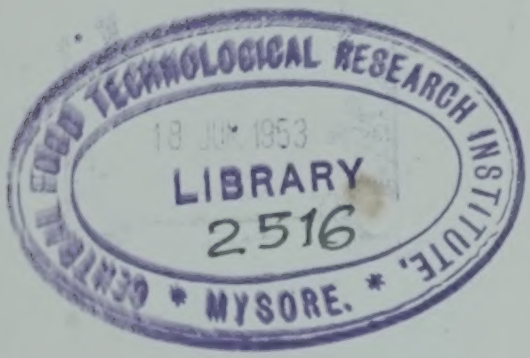

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The author dedicates

this book

to

JOHN U. BARR

of

New Orleans, La.

In grateful recognition of his work

in

Linking Industry with Science

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PREFACE

The purpose of this publication is to bring together in one volume the voluminous literature on the microbiology of sugar and sugar products, so that the sugar technologists may have a convenient source of references available which are applicable to problems connected with fermentation in sugar manufacture and the deterioration of sugars or syrups and molasses.

The writer wishes to acknowledge his indebtedness to various authors and sugar companies, whose collaboration has made this compilation possible. He wishes to express his very sincere appreciation to Dr. George P. Meade, General Manager of the Gramercy Refinery of the Colonial Sugar Company in Gramercy, Louisiana, for his kindness in reviewing the manuscript and offering suggestions as to the incorporation of material which had been overlooked, and to Dr. C. S. McClesky, Professor of Bacteriology at the Louisiana State University, for similar services. Also, to John Wiley & Sons, Publishers, who kindly permitted the use of data and drawings from the Eighth Edition of the Spencer-Meade Cane Sugar Handbook, and in addition he wishes to gratefully acknowledge the kind services rendered by other workers in this field, whose publications have been extensively reviewed in this publication. He is also immeasurably indebted to all of those who so generously subscribed to the pre-publication notices issued far in advance of the publication of this volume, for without this generous response on the part of those who are interested in this subject, its publication could not have been undertaken. The writer is also immeasurably indebted to the various Bureaus of the

U. S. Department of Agriculture, whose publications have been liberally referred to and quoted in this volume.

Since a publication of this kind has such a limited demand, the returns from sales rarely, if ever, warrant the cost of publication and it has only been through the generosity of Mr. John U. Barr of New Orleans, Louisiana, who is interested in all industries in Louisiana, especially the Sugar Industry, that the publication of the First Edition of this volume has been rendered possible and the writer wishes to express his very sincere appreciation for his generosity in this connection, an appreciation which is certain to be shared by all those in the Cane Sugar Industries in the World who are interested in the subject.

Wm. L. Owen

Baton Rouge, La.
May 10, 1949

Chapter I

SOURCE OF MICROORGANISMS IN CANE AND BEET JUICES

The origin of the microorganisms which normally constitute the microflora of both cane and beet sugars and the intermediate product, syrup, and the final product, molasses, may be classified as follows:

1. Those species of microorganisms which constitute what is known as the "epiphytic flora" of the sugar cane and sugar beet.

2. Those species of microorganisms which may have originally come from the soil, but which are propagated in the refinery or raw sugar factory and whose habitat is in the materials that always are to be found there, such as syrups, molasses or cane juices.

3. Microorganisms constantly being introduced into the factory or refinery from the dust in the air.

4. Microorganisms, especially thermophilic bacteria, that are frequently introduced into refineries in the starch which is used for the production of confectionery sugars.

The microorganisms that are derived from both cane and beets, exclusive, of course, of the soil particles which adhere to the outer surfaces of

this raw material, represent what may be called the "epiphytic flora", which is characteristic of all growing plants. This indigenous flora of sugar cane has been investigated by a number of investigators and the experiments of Wolzogen Kuhr (1) and Prinsen Geerligs (2) have amply demonstrated that the sugar cane carries with it a definite and specific bacterial flora. The first named investigator made a study of the bacterial flora of juices extracted aseptically from healthy sugar cane and of 597 samples examined only 6 yielded a sterile juice. These six samples, incidentally, were from cane grown in isolated mountain districts, where presumably the incidence of dust-borne bacteria was reduced to a minimum. All of the other samples showed a typical microflora, consisting, namely, of saprophytic bacteria, among which a chronegentic species, resembling Bact. herbicola a.aureum, was found.

Geerligs' publication does not reveal the nature of the microflora on cane, but only indicates the constancy of the presence of an indigenous bacterial flora from the juice aseptically extracted from cane.

Other investigators, namely Hutchinson and Ramayyar (3), isolated from fresh cane a yeast of the Saccharomyces cerevisiae type and Aspergillus species, but it is suspected that the origin of these species was either from the soil adhering to the cane, or from wounds of the cane stalk inflicted by cane borers.

In connection with the assumption, and the data in support of it, regarding the existence of "epiphytic" flora on all crop plants, it is interesting to refer to the extensive investigation publication made by Burri (4) and by Duggeli (5) on the additional flora on cereal plants. Duggeli

reported, in his investigation, the following concentration of bacteria per gram of different cereals, namely:

Oat Grains	1,330,000	bac.	per gram
Barley Grains	1,600,000	"	" "
Wheat "	500,000	"	" "
Rye "	126,000	"	" "

Hoffman's (6) investigation of the flora of Russian, Rumanian and German grains gave even higher figures, but inasmuch as the latter investigator included the flora of both high and low grades of cereal, the former data is regarded as the more authentic representation of the indigenous flora of cereal. The species constituting the "epiphytic" flora of cereals consist predominantly of short bacterial rods and, here again, the same species of Bact. herbicola a. aureum seems to be predominant. This species is synonymous with Mesentericus aureus Winkler (7) and described by Beijerinck (8) as B. anglomerans.

Both in cane and beet sugar agronomy, as opposed to that of cereal crops, the epiphytic flora of these plants receives annual additions of microorganisms, more or less acclimated to the products of the sugar factory, since filter-press mud from the factories or refineries is normally returned to the fields. As a result of this procedure, the flora of these soils, where these sugar crops are planted, is continually being enriched by the introduction of materials from the sugar factory, which contain not only a very large variety of microorganisms, but species which have been acclimatized, to some extent at least, to the development of sugar substrates of various concentrations. In a similar manner, the transportation of cane or beets from fields to the factories and the return of these wagons, trucks or railway cars

to the field undoubtedly result in a constant interchange of the microflora from the sugar factories to the fields and vice versa. Under these conditions, it is easy to conceive of both cane and beet having a more distinctive "epiphytic" flora than would be true of other crop plants.

In addition to these sources of microorganisms in sugar products, there are, of course, the microbial contaminants in the dust of the factories or refineries, where under ordinary conditions a rather high concentration of those species of microorganisms capable of growing in sugar products are being constantly developed. It is reasonably certain, too, that in this constant exposure of these species to sugar solutions of various concentrations and the successive drying of these products on the floors and walls of the building, some of these species acquire distinctive adaptabilities to substrates of high sugar concentrations. This certainly is true of certain spore-forming soil bacteria, exemplified by several of the strains of Bacillus mesentericus and vulgatus. These species, as isolated directly from soils, lack the property of producing gum levan from sucrose, whereas the acclimatized species of these bacteria, when isolated from sugar factories and sugar products, have this acquired character. The writer has shown that by continuous transfer of the soil type of these species into sucrose solutions of varying concentrations, the ability to form gum levan is readily acquired.

While it is generally assumed that the freshly extracted juice from canes or beets, which have not been injured and whose rinds are intact, contain relatively few microorganisms, when these rinds have been punctured by borers or other insects they frequently yield juices which have a very high incidence of microorganisms. In the

cases of cane plants which have been damaged by the cane borer, the microbial content of the extracted juice may be relatively high in bacteria, yeast or fungi. Among the normal inhabitants of these borer wounds is a bacterial species which was first isolated by the writer (9), and to which he applied the name of B. saccharalis. This species is of great scientific interest, in view of the fact that it has the property of producing ethyl alcohol from glycerin. It is commonly found in borer infested canes, but its economic importance in that habitat has not yet been established. It is obvious that where infection of sugar cane with the cane borer is very high, the extracted juice from it will carry a relatively high concentration of microorganisms.

McKaig and Fort (10), in their study of the composition of juice from Louisiana sugar cane, injured by the borer and the red rot disease, found a decrease in the percentage of solids and sucrose in the juice and an increase in the total organic non-sugars and both the protein and non-protein in nitrogen were also increased. All the mineral constituents in diseased cane were higher than in normal cane of the same variety with which they were compared. Iwata (11) also found that the juice of cane injured by disease or insect pests contains more nitrogen and has a lower pH than normal juice.

Of the microorganisms brought to the sugar factory from the field, we may quite readily expect the Leuconostoc mesenteroides to be included, although its presence has not been established either in the soil from cane fields or as a constituent of the "epiphytic" flora of cane.

Its presence is rarely detected in sugar factories or in sugar products, until the cane or

beet is damaged by freezing, although the presence of some varieties of this species may be detected in the form of small clumps of gelatinous material around the mill and particularly on the juice strainers, even under normal operation. It is more than likely that this species does constitute definite parts of the indigenous flora of both cane and beets, particularly since frozen canes in the field may almost spontaneously undergo this type of fermentation, especially when the rinds have been split by severe freezes.

It is evident that the first of these groups is continually brought into both cane and beet factories, both from the soil and cane and beets themselves. However, the interior of most factories of this kind is so continuously contaminated from the air and from dust, incident to processing operations, that the additional contaminants that may be brought in with the raw material are of no great importance, but yeast contaminants are probably autogenous to the factory itself, in which there are almost always zones of contamination from fermenting juices and dilute samples, or wash waters, and as this material becomes dry from air currents, yeasts are consistently present in great numbers in the air of such plants. The thermophilic bacteria, being of the sporogenous species, may constantly occur in air-borne dust in such plants and in refineries, where their presence is of economic importance, and it has been found that starch dust is one of the principal sources of this kind of contamination. Under conditions of raw sugar manufacture, either from cane or beets, it is still a debatable question as to whether any significant losses of sucrose may be attributed to microbial action. The time intervening between the extraction of the juice from the cane and its exposure to high temperatures of clarification reduces opportunities for microbial development to

very short intervals and there have been few reported instances where detectable losses of sugar have occurred during this interval. McCleery (12) reported a considerable reduction in the drop of purity between crushes and mill juices, as a result of mill sanitation and frequent mill cleaning and the use of steam at frequent intervals. This would indicate that the loss of sucrose during milling is of considerable significance, if some precautions are not taken to maintain sanitary conditions in the main mill.

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Chapter II

MICROBIAL FLORA--SUGAR CANE AND BEET JUICES

From the foregoing brief references to the sources of microorganisms in cane and beet sugar factories, we can assume that even under the best harvesting conditions the freshly extracted juices from these plants will contain a rather definite "epiphytic" flora and even where there is no material contribution from this flora to the sugar mills and the beet slicer, the juices from both of these products will be fairly highly contaminated when freshly extracted. It may be assumed, moreover, that except for the presence of the dextran-forming bacteria in cane juices under normal conditions the concentration of microorganisms in these products are of no great economic significance under normal operations. The consequences of such contamination, however, may be quite real in the event of shutdowns in the factories, necessitating the holding of the juices of the cane or the diffusion juices from beets for any considerable length of time. The question of what is the normal microbial concentration in freshly extracted cane juice is yet to be adequately answered, because such an appraisal requires a variety of specific procedures, each of which is designed to reveal the presence of specific constituents of the microbial flora of these products. For example, one would have to make one assay for the determination of mesophilic bacteria present, another for anaerobic bacteria present, still another for the number of viable mould spores and still another for the determination of thermophilic bacteria and a special procedure would also be required for the determination of the viable yeast

present. Such an elaborate appraisal of the normal flora of either cane or beet juices has not, as far as the writer is aware, yet been made. In some early investigations by the writer, an appraisal was made of the mesophilic bacteria in raw juices and refined products, but at that time the essential role of certain growth factors in bacteriological substrates was not known and, hence, the results then reported did not present an adequate picture of the degree of contamination of those products. We are indebted to Millstein, Tobin and McClesky (1), of the Department of Bacteriology at the Louisiana State University, for some more recent data on the concentration of viable bacteria in normal cane juices, which shows the very high levels of viable bacteria occurring in normal freshly extracted juices. These same authors have given us some recent data, also, on the concentration of viable thermophilic bacteria in raw juices and their occurrence in various stages of the sugar manufacturing process.

As stated in the previous section of this chapter, it still remains a debatable question as to whether under normal operating conditions there are many material losses in sucrose, due to microbial activities. McAllep (2) has shown that juices held over for 18 hours at a temperature varying between 180° and 190° F. lost an average of 0.21° in final purity and Carpenter and Bomonti (3) show decreases in boiled juices amounting to as much as 2.47° in a period of 42 hours. The results of some of the experiments of the latter investigators are as given in the table on page 10.

These authors isolated a thermophilic species from juices, to which they gave the name Microspira Northii; however, they did not report any data resulting from the inoculation of sterile cane juices with this organism, so the economic importance of

DETERIORATION OF BOILED JUICE AND STERILE JUICE

	50° C.				60° C.				70° C.			
	Boiled Juice		Sterile Check		Boiled Juice		Sterile Check		Boiled Juice		Sterile Check	
Hours	Purity	De-crease	Purity	De-crease	Purity	De-crease	Purity	De-crease	Purity	De-crease	Purity	De-crease
0	88.94	...	86.65	...	88.94	...	86.65	...	88.94	...	86.65	...
18	88.95	+ 0.01	86.33	0.32	88.35	0.59	85.89	0.76	88.55	0.39	86.15	0.50
42	86.47	2.47	85.63	1.02	84.47	4.47	85.34	1.31	83.83	5.11	83.82	2.83

this species, in connection with the deterioration of sugar products, has not been established.

There is a great deal of evidence to support the assumption that the large percentage of microflora of freshly extracted cane juice is attached to particles of bagasse and bagacillo, which is always present in mill juices. As evidence of this fact, the reports of the authors may be cited, in which bacteriological analyses were made of raw juices and juices which had been filtered over various clarification media, including Diatomaceous earth and various activated carbons. The averages of a large number of such tests show that merely filtering juices through absorbent cotton removed an average of seventy-five percent of the microorganisms originally present and the usual absorption agent used in clarification removed over ninety-nine percent of the microorganisms originally contained in the juice. A summary of this data is shown in the table on page 11.

It will be noted that the percentage of removal of the microorganisms in raw juices by filters, is not at all parallel with the increases in purity brought about in this procedure. The assumption that the concentration of microorganisms occurring in freshly extracted cane juice is

Sample	Average Increase in Purity	Average Microorganisms removed percent
Filter cotton	...	75.1
Filter-cell	2.7	99.69
Darco	3.99	99.54
Norit	1.70	99.45
Suchar	4.83	99.40

intimately associated with the mechanical impurities of these products is further indicated by the results of investigations carried out by the Hawaiian Sugar Experiment Station, in which the drop in purity between the crushed and mixed juices was as follows.

> Over a 15-weeks period of grinding with a nine-roller mill, where the chances for fermentation were quite low, the average difference between crushed and mixed juices was 2.75, whereas over a 20-weeks period of grinding, with eighteen mills, these differences were 4.16 and 4.53.

The authors claim that the above results caused them to wash down the mill more frequently and, on some occasions, the mill was stopped purposely to clean it with hot water. On days immediately after cleaning the mill, the difference between the purities of crushed and mixed juices averaged 2.07, but thereafter it averaged 3.07.

THE MILL STERILIZATION IN SUGAR CANE MANUFACTURE

Very recently, procedures have been described for the sterilization of mill juices at the time of their extraction and the Daniel Sterilization Process (18), using chlorine, is claimed to increase the purity of the juice by the prevention of microbial growth and it will be noted in the following table that the purities of both the crushed and mixed juices were higher. The authors claim that a gain of 0.54 in the purity of the mixed juice represented an increase in sugar recovery of 31,250 pounds for each million arrobas of cane ground.

	Without Chlorine Tandem	With Chlorine Tandem
	"B"	"C"
Purity crusher juice	87.84	87.31
Purity mixed juice	84.77	84.32
Purity mixed juice, 5 minutes more	84.40	84.52
Purity mixed juice, 10 minutes more	84.12	84.41

The authors cite the data from the investigation by Pederson (19) on the level of microbial population in freshly extracted cane juice and it will be seen from this data that the level of population in unsterilized and unheated cane juice may be exceedingly high. It seems entirely consistent that a decrease of this number of microorganisms by effective sterilization procedures would result in the prevention of sugar loss through fermentation. The following table shows microscopic counts of juices extracted from stored, cut canes.

No. of Samples	Source	Microscopic Count
11	First mill	3,900,000
45	Crusher trough and 1st mill	27,000,000
40	Trough 3rd and 4th mills	275,000,000

MICROSCOPIC COUNTS OF GELATINOUS RESIDUES UNDER CRUSHERS AND MILLS

No. of Samples	Source	Microscopic Count
13	Under 1st crusher	38,500,000
15	Under 1st mill	58,000,000
42	Under 2nd and 3rd mills	78,000,000
51	Under 2nd mill	500,000,000

MICROORGANISMS IN BEET JUICES

We are indebted to Schone (4) for his very extensive study and investigations of the microflora of these products. This author found the microflora of cossettes and the fresh sugar beet diffusion juices was 860 and 4,200 per gram of cossettes, supplied by two different factories and a count of 1,481,000 as the average count per c.c.m. of raw diffusion juice by four different factories. Of the species of bacteria isolated by this investigator, may be mentioned Leuconostoc mesenteroides, Bacillus mesentericus, Bacillus subtilis, Bact. prodigiosum and Bact. fluorescens liquefaciens. Thaysen and Galloway (5) called attention to the difference between Schone's figure on the bacterial content of cossette and diffussion juices and suggested that this infection was probably increased by impure water used for extraction.

Prior to the investigations of the above author of the microbial content of beet juices in diffusion batteries of beet factories, Orth (6) found bacterial concentrations of as high as 200,000 per C. C. and Laxa (7) observed even higher concentrations of bacteria in the diffusion juices in two factories, the predominant species of which was sporogenous and the most prominent one of which was Clostridium gelatinosum. It is interesting to observe that Schone detected bacterial growth in the juices of the diffusion batteries up to a temperature of 75°C. Even higher levels of bacterial population in the freshly filled diffusers were observed by Raschkowtisch (8).

MICROORGANISMS IN RAW CANE JUICES

Browne (9) observed, in connection with his study of the fermentation of sugar cane products, that sugar cane, in common with all plant juices, exercises a definite bactericidal action for some hours after it is extracted. This protectiveness against microbial growth and development he attributes to the oxidases which this product contains. His data on the oxygen absorption of fresh and boiled cane juices is as follows:

CUBIC CENTIMETERS AIR ABSORBED

	1st day	2nd day	3rd day	4th day	Total
Fresh juice	16	13	7	1	37
Boiled juice	4	5	4	3	16

His data on the bactericidal action of freshly extracted cane juice is shown in the following table:

Experiment	Source of Juice	Temp.	Number of bacteria per cubic centimeter of juice				
			At beginning	In 4 hrs.	In 8 hrs.	In 24 hrs.	In 36 hrs.
1	Green Top	10°	2,761,920	294,336	262,080	211,800	.....
1	Green Top	10°	2,681,280	241,920	231,840	187,488	.....
2	Green Top	15°	106,880	96,320	98,240	.....	.....
3	Frozen Cane Top	15°	260,160	274,560	320,000	960,000	.....
3	Sound Cane, bottom	15°	458,880	465,600	475,840	560,000	.....
4	Steamed Cane	15°	276,800	308,160	405,120	985,600	.....
4	Raw Cane	15°	101,760	94,180	130,240	280,000	.....
5	Steamed Cane	20°	12,480	20,160	33,600	311,040	3,840,000
5	Raw Cane	20°	37,440	43,840	65,280	902,400	5,120,000

There would seem to be no doubt that freshly extracted cane juice exercises an inhibitory action upon the development of microorganisms, for some hours after its extraction from the cane, that is, if the cane is not frozen and if free of infestation with borers or other insects. Of course, this property of cane juice has to be determined under aseptic conditions, owing to the fact that during milling the freshly extracted juice is constantly acquiring a large microbial population from the accumulation of contaminated particles of bagasse in the milling trains and in the juice trains. It will be noted in the table on page 15 that in the juices from frozen cane the bacterial growth was continuous and no depressing action was observed in the early period of observation.

In connection with the consideration of the growth of microorganisms in freshly extracted cane juices and the germicidal power of freshly extracted juices, resulting from plant enzyme action, as pointed out above, it is generally known that the juices from burned cane offer considerable difficulties, as regards their clarification. The practice of burning cane to remove adhering trash is generally practiced in Hawaii and, to some extent, in Cuba. In addition, moreover, to the canes that are brought to the mill, which have been intentionally burned to facilitate harvesting, there frequently occurs spontaneous fires in cane fields in Cuba and in Puerto Rica, which sometimes cover large acreages. It is generally recognized in the Industry that burned cane must be harvested very promptly, to avoid losses from deterioration, which, in this case, progresses very rapidly. Meade (10) reports that the Cuban cane contracts, covering the purchase of burned cane, include the clause that no penalty in prices is applied within five days after the cane has been burned, that is if the cane has not been exposed to rain, in the

meantime. However, where it has been so exposed such canes may be rejected at the discretion of the buyer. Whether the deterioration of the cane is entirely the result of autogenous enzymatic action, or whether the exposed juice deteriorates more rapidly as a result of the growth of microorganisms, has not, so far as the writer is aware, been definitely determined. It may be presumed that a great deal of it is purely autogenous, but it may well be that the inactivation of the enzymes in the juice may render the product much more susceptible to the growth and activities of microorganisms.

CLASSIFICATION OF MICROORGANISMS THAT HAVE BEEN ISOLATED FROM CANE AND BEET JUICES

The groups of microorganisms that have been isolated from cane and beet may be outlined as follows:

I. BACTERIA
- A. The causative agents in viscous fermentations of cane and beet juices.
  - 1. Dextran formers.
    - a. Leuconostoc mesenteroides (Van Teighem - amend. Hucker & Pederson)
    - b. Bact. pediculatum (Koch and Hosaeus)
    - c. Bact. vermiforme (Ward)
    - d. Bacterium gelatinosum betae (Glaser)
    - e. Semi-Clostridium commune (Maasen)
    - f. Semi-Clostridium citreum
    - g. Semi-Clostridium flavum
    - h. Semi-Clostridium rubrum
    - i. Mycococcus betae
    - j. Clostridium gelatinosum (Gonnerman)
  - 2. Levan formers
    - a. Levaniformans (Greig-Smith)
    - b. Mesentericus vulgatus (Owen)

c. Mesentericus fuscus (Owen)
d. Mesentericus liodermos (Owen)

3. Acid formers
a. Lactobacilli
b. Aceto bacter xylinium (Browne)

II. Miscellaneous bacteria, both from the epiphytic flora of the cane and beet, together with soil contaminants brought to the factories with the harvested plants. None of the species have, as yet, been found to have any significance in the deteriorative changes of cane or beet.

a. B. subtilis
b. B. megatherium
c. B. mycoides
d. E. coli communis
e. Proteus vulgaris

III. MOLD FUNGI

A. The species of mould fungi that have been isolated from sugars by the Kopeloffs (11) Church and Thom (12), Browne (13), Ammons (14), Van der Bijl (15), and by the writer (16) are presumed to occur in the raw juices of the cane and to be derived from the trash and the dust adhering to the mill cane. However, authentic data is lacking as to the normal mould population of cane juice as freshly extracted, which in all probability, varies with the cleanliness of the cane and the amount of dust that it contains. We can feel safe in assuming that the role played by fungi, in the deterioration of juice during the brief interval that elapses under normal mill operations, between the extraction of juice and its exposure to lethal temperatures, incident to clarification, is insignificant, owing to the fact that the time

required for the germination of the spores would ordinarily be too prolonged to provide zymogenically active vegetative mycelia prior to the clarification procedures.

B. Fungi Isolated from Cane Sugars

1. Series I
   a. Aspergillus niger
   b. Aspergillus flavus
   c. Aspergillus nidulans
   d. Blue Aspergillus
   e. Citromyces I, II, III
   f. Penicillium expansum (?)
   g. Penicillium divarticatum
   h. Penicillium luteum ser.
   i. Syncephalastrum
   j. Trichoderma sp.
   k. Sterile Orange fungus
   l. Unknown I, II, III, IV
2. Series II
   a. Aspergillus sp.
   b. Aspergillus Bur. Chem No. 3556
   c. Aspergillus repens
   d. Penicillium purpurogenum ser. near Penicillium pinophilum
   e. Penicillium luteum, a soil form
   f. Penicillium luteum - purpurogenum ser. near Penicillium regulosum
   g. Fusarium sp.
   h. Monilia nigra (Browne)
   i. Monascus purpurens ser.
   j. Dematiaceous fungus
   k. Unknown V, VI, etc.
   l. Penicillium roseum

IV. YEAST

Although the Saccharomyces constituent of normal freshly extracted cane or beet juices has not been subjected to any extended investigation, we may assume that the species

found in raw sugars probably originated in the raw material brought into the plant. Taking this as a basis for our appraisal, together with species isolated from juices, which are converted into rum, by spontaneous fermentation, we may assume that the following species are constantly present in the raw juices. (See table on following page)

In addition to the above species of yeast that have been isolated from cane sugars and cane molasses, cane juice has proven a prolific source for the best strains of yeast for the manufacture of rum from molasses and Ashby (17) has listed a large number of both Saccharomyces and Zygosaccharomyces from this source.

CONCLUSION

From these observations and a review of all of the available literature on the subject, it may be concluded that both in cane and beet sugar manufacture the raw material brought into the factory for fabrication has a very high total microbial population, which increases proportionally with the amount of soil and trash that adheres to the cane or to the beet and that this population increases with the conditions of the fields from which the plants are harvested, thus in rainy weather a great more soil adheres to the plants and thereby greatly increases the initial bacterial levels in the freshly extracted juices. In the cane sugar industry, it is accentuated more by the use of cane harvesters and by the increasing amounts of cane trash that now adhere to the cane. This problem of trashy cane is becoming more and more serious almost everywhere and undoubtedly the problem of sanitation in raw sugar factories is becoming more and more acute.

Species	Source	Described by	Reference
Cryptosaccharomyces [illegible]	Barbados Molasses	Hall, Owen & Nelson	Food Res., Vol. 7, No. 6, 1942
Cryptosaccharomyces major	Barbados Molasses	Hall, Owen & Nelson	Food Res., Vol. 7, No. 6, 1942
Torula [illegible] (Browne)	Cane Sugars	Browne	J. Ind. & Eng. Chem., Vol. 10, No. 3, Pg. 175, 1918
Saccharomyces zopfii (Artari)	Cane Syrups	Owen	Cent. fur Bakteriologie, 1913
Schizosaccharomyces (Pombe Lindner)	Refinery Cane Molasses	de Whalley & Scurr	Chem. & Ind., Aug. 30 1947
Cryptosaccharomyces globiformis	Refinery Cane Molasses	de Whalley & Scurr	Chem. & Ind., Aug. 30 1947
Torulae Strains (20 strains)	Raw Cane Sugars, differentiated by morphological and physiological characterization	Owen	
Schizosaccharomyces malleyi (Jorgensen)	Molasses mashes used in production of rum in Jamaica	Greg	Bull. Botanical Dept. Vol. 2, 1895

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Chapter III

TYPICAL FERMENTATIONS--CANE AND BEET JUICES

Both cane and beet juices are ideally suited for the development of a wide variety of species of microorganisms, owing to the fact that

1. they contain an abundance of nutrient material,
2. their density is at appropriate levels for the growth of most microorganisms,
3. they are well endowed with growth factors and vitamins and
4. under normal conditions, their pH is at optimum levels for a large number of species of bacteria, yeast and mould fungi.

According to the data reported by Fort and McKaig (1) of the U. S. Department of Agriculture, the nitrogenous bodies of cane is 0.40%, of which albuminoids, amido acids and amides predominate. These authors also found that the pH of juices from various sources were as follows:

	Average pH
Louisiana crushed juice	5.38
Variation in individual samples	5.22-5.63
Florida	5.17

Paine and Balch (2) found the following levels of pH for juices from Puerto Rica and the Philippines:

Puerto Rico averaged 5.29 with a range of 4.73-5.29
Philippines averaged 5.39 with a range of 5.26-5.57

As to titratable acidity, Fort and Lauritzen (3) found it varied from 1.5 to 3.0 C. C's N/10NaOH (10 C. C's of juice) and an extreme variation for Louisiana juices was from 0.7 to 4.5. The correlation between excess acidity in cane juices and the extent of deterioration of the juices by microbial action is well recognized and the use of titratable acidity, as a means of appraising the extent of deterioration, has become a well recognized practice in the Sugar Industry.

THE CORRELATION OF JUICE ACIDITIES AND THE DEGREE OF CANE INJURY FROM FREEZES

In canes damaged by freezes the acidity values increase and the extent of this increase may be correlated with the degree of injury the cane has suffered and the clarification problems that it will present in the recovery of sucrose from it. We are indebted to the above authors for their extensive investigation and their published data on this problem and the conclusion which they reached in their extensive study was that an excess of acidity, corresponding to more than a 0.4 pH, is regarded as being indicative of damaged cane. These authors claim that the variation over the whole area is on the average of a difference between 5.2 and 5.6 pH. Values below 5.2 they claim are due to excess acidity. If the excess acidity is as great as 1.0, it is believed that the juice is unfit for factory use. They developed a distillation method based upon the assumption that most of the excess acidity produced was

acetic, which could be steam-distilled from the juice. Their method consists in taking a 100-cc. juice sample and introducing it into a 300-cc. flask, connected with a condenser. From this sample, 25 cc's of distillate are recovered and titrated with 0.1 N alkali, using phenolphthalein as indicator. The value obtained was found to be a fair approximation of the excess acidities of 10 cc's of juice, that is approximately 10% of the total excess acidity of the 100-cc. sample distilled over in the first 25 cc's of condensate, which is equivalent to the excess acidity in 10 cc's of juice. Some of these results are given herewith:

	Initial Acidity	Total Acidity After Souring	Excess Acidity
	Cc.	Cc.	Cc.
Whole cane (Normally topped)	2.21	3.50	1.29
Cane topped back one-third (average of bottom two-thirds)	1.58	2.69	1.11
Top third section	2.74	5.60	2.86
Middle third section	1.86	3.48	1.62
Bottom third section	1.30	1.90	0.60

VITAMINS IN CANE JUICES

The vitamins in cane juices have not been fully appraised up to this time, although the scientific literature on the subject is already quite extensive. Asikaga (4) has found considerable ascorbic acid in the leaves of immature cane

and Biswas (5) has reported yields from cane molasses as high as 10.4 units of B1 and 2.0 units of B2. Nelson and D. Breese (6) have reported that cane juice contains some Vitamin A, little, if any, Vitamin D and that the upper or less mature portion of the cane is richer in Vitamin B than the more mature portions. Funk and Freedman (7), on the contrary, have shown that refined cane sugar itself contains a vitamin-like substance that stimulates the growth of yeast cells and is probably identical with Vitamin D. Nelson, Heller and Fulmer (8), who studied the comparative Vitamin B_1 content of sorghum cane blackstrap and beet molasses, found that the cane blackstrap was the richest of the three and that sorghum contained practically none. More recently, 1938, Yamafuji and Ohtsu (9) have reported that cane juice possesses unusual properties as a yeast nutrient material, in which respect it proved superior to either malt or Koji decoctions. While the last named investigators did not employ methods that would have differentiated the factors of nutrient contribution by the cane juice employed and the presence of vitamins or growth factors, it is unquestionable that this material possesses vitamin value which is progressively removed during the process of sugar or syrup manufacture.

Jackson (10) reported the following vitamins in cane juices:

	Whole Cane		Juice	
	Minimum	Maximum	Minimum	Maximum
Thiamine	2.05	17.95	0.50	1.1
Riboflavin	1.1	5.7	0.215	0.4
Pantothenic acid	6.0	38.6	4.4	9.9
Niacin	5.3	52.25	2.0	3.3
Biotin	0.2	0.7	0.08	0.1

TYPICAL SPONTANEOUS FERMENTATION OF CANE JUICE

From the foregoing description of the qualitative and quantitative microbiological bacteria of cane juice, as freshly extracted, we may assume that it contains a sufficiently high potential of microbial agents to induce prompt and rapid decomposition of its sugars. Contrary to the genera belief, the most usual and most universal fermentation that takes place in a freshly extracted can juice is that of alcoholic fermentation. Browne (11) regarded a reducing fermentation, as exemplified by the formation of dextran and mannite, as being the most common in Louisiana juice. Schone (12) expressed the opinion that even where the acetic nature is unfavorable for the production of dextran by L. mesenteroides that organism is still capable of inverting sucrose. He attributes much of the loss of sucrose of juices as resulting from microbial contamination to the growth of the above organism in an uncapsulated form. The explanation of the apparent frequency with which juices undergo alcoholic fermentation by yeast, as compared with that from bacterial development, is to be found in the condition in which the "epiphytic" flora of cane exists. By far, the larger number of species in bacteria which are found in freshly extracted juices are spore-forming organisms which can survive in extreme dessication and are to be found in dust and in the dry leaves of the cane. These are immediately transferred to the extracted juice, but the time required for the spore germination is, even at optimum temperature, a matter of at least 24 hours. On the other hand, yeast contaminants, although they may be in a semi-dessicated condition, absorb moisture much more rapidly and, hence, germinate in a much shorter period than do the spores of bacteria. It is true that a rather large number of asperogenous species of bacteria are found in freshly extracted juices

and among the most important of these are Leuconostoc mesenteroides, whose habitat is suspected as being on the cane or around the premises of the sugar factories, although this is yet to be definitely proven, but even conceding that it exists in a highly dehydrated condition in dust it is seldom that the reaction of juice is sufficiently near the neutral point to make it as suitable for this type of fermentation as for the alcoholic. We do not mean to imply that any specific type of fermentation ever monopolizes the fermentation of cane juice, even during the short period of time which intervenes between the extraction of the juice and its heating, incident to clarification. Even in this limited time, the fermentation is almost universally the heterogenous resultant of various groups of microorganisms competing for predominance. For example, the initial stages of alcoholic fermentation contributes by its reducing action to the activities of the Leuconostoc mesenteroides, which is favored, to some extent, by the absence of oxygen. Indeed, there are several gum-forming species of bacteria which grow with yeast by the action above referred to.

The illustration given above, as to the spontaneous development of the various assortments of microorganisms in juices, and the explanation for the greater frequency of alcoholic fermentation, as induced by yeast, would apply, of course, only to mills which are in a sanitary condition, that is to say free from fermenting particles of bagasse or cane trash saturated with juice and highly impregnated with microbial contaminants. Under these conditions, we may have, as contaminants, active vegetative cells developing from spore-forming bacteria, as well as vegetative cells of yeast and fungi. We can readily see that the time factor for the germination of bacterial spores is no longer applicable and, hence, there would be

a parallel growth of all of the classes of microorganisms for which cane juice is suitable as a substrate. Coincidentally with the formation of alcohol, there is an oxidation at the surface by acetic acid bacteria and film yeast, which explain the usual acetic odor that is so frequently detected around sugar factories.

It is obvious, both from the composition of normal cane juice, with reference to its nutrient content, its reaction and the presence of growth factors, that it is almost an ideal substrate for a wide variety of microorganisms, in fact it is so rich in growth factors that wherever it is possible to retain the fresh product, it is profitably employed in varying amounts in ordinary bacterial substrates. Immediately the juice is extracted and where the prevailing temperatures are favorable, as is almost universally true throughout the cane sugar producing areas of the world, the development of microorganisms takes place almost immediately. In view of the heterogenous mixture of microbial contaminants, we have, as a rule, the following microorganisms competing for supremacy in this material, namely:

1. Various species of Saccharomycetes. (Both true and false yeasts)
2. Mesophilic bacteria
   a. aerobes
   b. anaerobes
3. Gum-forming bacteria, namely
   a. Leuconostoc mesenteroides (dextran-forming bacteria)
   b. Bacillus mesentericus (levan-forming bacteria)
   c. Butyric acid bacteria
   d. Lactobacilli

Under ordinary conditions, it is generally assumed that in the time that intervenes between the

extraction of the juice from the cane and its transfer to the clarifiers, no perceptible loss in sucrose occurs; however, there is strong evidence that this does not apply to milling operations, where the mill is not maintained in a sanitary condition. For example, Orth (13) observed that under unfavorable conditions, resulting from the operation of dirty mills, the loss of sucrose was quite rapid. On the other hand, when cleanliness was maintained the juice from the last mill could be kept for five hours with little signs of deterioration taking place. These observations by Orth were confirmed by Sprankling (14), who records an instance where cane juice, standing for fourteen hours, lost 14.3% of its sucrose in six hours, or 0.04% per minute.

THE VISCOUS FERMENTATION OF CANE JUICE

Of all the spontaneous fermentations which cane juices undergo, the dextran, or viscous fermentation is, by far, the best known and the one which has been most extensively studied. As early as 1822, the occurrence of gum dextran in some bottles of cane juice, shipped from Martinique to France, attracted the attention of Vauquelin (15), who attempted to determine its chemical composition by analysis. Later Durin (16), who investigated and submitted the gum to chemical analysis, concluded that it was cellulose and applied for a patent for the conversion of sucrose into cellulose by a fermentation process. It remained for Scheibler (17) to establish the true nature of the product and to give it its name, dextran, owing to its similarity to dextrin. We are indebted to Browne (18) for his review of the literature on the viscous fermentation of cane juices and for his extensive researches on the chemical identification and establishment of the chemical formula for the purified product, as it occurs in spontane-

ously fermented cane juices, and for its effect upon the polarization of sugar products. More recently, McCalip and Hall (19) and Walton and Fort (20) have studied the occurrence of the gum in juices from frozen cane and its effect upon the appraisal of the sucrose value of such juices, and Walton and Fort (21) the jellying effects of dextran and the occurrence of mannite in molasses produced from frozen cane. The first of these above investigations is of special significance, inasmuch as it provides the first study, as far as the writer is aware, of the rate of dextran formation in sucrose solutions ideally enriched and buffered to provide optimum conditions for the growth of the organisms. Their results are given in the following table: (See table on page 33)

It will be seen from the above table that the formation of dextran from sucrose, by Leuconostoc mesenteroides, under ideal conditions is very rapid and that the acidity increases are directly proportionate thereto. The error that the presence of the dextran introduces into the sucrose determinations of juices, by the conventional procedure, is shown in the following table from McCalip's and Hall's (22) publication, from which it will be noted that using the Hornes Dry Lead Method, which is the conventional method for the appraisal of sucrose in juices, a juice from damaged canes might show a direct polarization of 52.68, when the true polarization after precipitation and removal of the gum would be only 13.10. (See table on page 34)

It can be well appreciated that even a trace of dextran, which has a specific rotation of 200, would greatly increase the polarization of juices in which they occur and, unless removed by special methods in the analysis, would introduce tremendous errors in the appraisal of sucrose and the purities of such juices.

EFFECT OF GROWTH OF L. MESENTEROIDES ON THE PROPERTIES OF A 10% SUCROSE SOLUTION

Sample	Refractometer Solids	Direct Sucrose	Invertase Invert Reading	True Sucrose by Invertase	Mw. of Invert Sugar 100 gm	pH	Titrated Acidity 1/10 N NaOH/10 cc	Mw. of Crude Gum	Viscosity Engler	Plate Count cc.
At time of inoculation	10.05	8.64	3.44	....	....	7.59	.18	3.1	1.07	23,000
6 hrs. after	.....	....	....	....	....	6.45	.25	3.6	1.12	40,000
24 " "	10.05	9.45	3.13	9.60	....	5.43	.39	9.8	1.26	50,000,000
48 " "	10.00	9.10	3.40	9.52	238	4.72	.45	12.9	1.66	39,000,000
72 " "	10.00	8.48	-3.30	8.97	467.7	4.25	...	....	1.502	.....
96 " "	10.00	8.80	-3.10	9.07	404.6	4.19	.55	37.0	1.394	16,000,000
144 " "	10.05	8.50	-2.70	8.53	577.7	4.08	.6	53.4	1.55	3,900,000
192 " "	10.05	8.85	-2.94	8.98	386.9	4.00	.61	48.9	1.62	1,600,000
240 " "	10.05	8.92	-2.94	9.04	433.8	4.16	.55	43.1	1.384	2,200
288 " "	10.00	8.62	-2.90	8.78	504.6	4.06	.55	59.3	1.612	3,600

COMPARISON OF RESULTS WHEN ANALYZING JUICE FROM SOUND AND DETERIORATED CANE, USING HORNE'S METHOD AND NORMAL WEIGHT METHOD CLARIFYING WITH AND WITHOUT THE USE OF ALCOHOL

Material	Acidity cc N/100 NaOH per 10 cc	Method	Method of Clarifying	Direct Polarization	Acid Invert Reading	Invertase Invert Reading	Direct Sucrose	True Sucrose	True Sucrose Invertase
Juice from windrowed cane	2.6	Horne's Method	Dry Lead	52.68	-20.34	-19.94	12.86	13.42	13.44
" " "	2.6	N/Wt.	L.S.A. Sol.**	12.76	- 4.84	4.76	12.76	13.31	13.34
" " "	2.6	N/Wt.	L.S.A.*** Sol. and Alc.	13.10	- 4.30	*-4.04	13.10	13.15	.....
Juice from deteriorated cane	8.7	Horne's Method	Dry Lead	35.35	- 3.22	-1.80	8.71	7.22	6.97
" " "	8.7	N/Wt.	L.S.A. Sol. and Alc.	7.25	- 2.00	*-2.34	7.25	6.98	7.28
" " "	7.7	Horne's	Dry Lead	42.5	- 6.64	-5.50	10.37	9.02	8.87
" " "	7.7	N/Wt.	L.S.A. Sol.	9.58	.....	-2.04	9.58	....	8.85
" " "	7.7	N/Wt.	L.S.A.	9.42	- 2.70	*-2.50	9.42	9.17	9.08

*Alcohol removed before invertase added
**Lead-sub-Acetate Solution
***Lead-sub-Acetate Solution and Alcohol

We are indebted to Alford and McClesky (23) for the first comprehensive survey of the species of dextran formers occurring in Louisiana cane juices. These authors found three types of dextran formers occurring in cane juices and these three were identified as L. citrovorus, L. mesenteroides and L. dextranicus, as described by Hucker and Pederson (24).

Contrary to the previous concept of the uniformity of the agents responsible for the spontaneous viscous fermentation of juices, Alford and McClesky (25) found that the species L. mesenteroides was far less efficient as a dextran former than was L. dextranicus and the latter appears to be of two types, one having greater gum producing power in raw and the other in refined sugar solutions. One group of L. dextranicus was differentiated by its characteristic ability to induce fluorescence in a substrate containing 5 percent or more of sugar. The authors found that none of the species of L. mesenteroides could solidify a 1% sugar solution, whereas all strains of L. dextranicus studied possessed the ability to produce sufficient dextran to effect this transformation.

THE SUSCEPTIBILITY OF JUICES FROM FROZEN CANE TO VISCOUS FERMENTATION

We have already referred to the rapidity and we might, with some reservations, state the ubiquity with which juices from frozen cane undergo highly destructive viscous fermentations, which induce endless difficulties in the clarification procedures and the chemical appraisals of the sucrose present in the mill juice. In spite of the spontaneity of these fermentations and their disastrous effects upon sucrose recoveries, we

have reason to assume that the potential production of viscosity arising from dextran fermentation is never attained and that the final viscosities and the sucrose destruction resulting, therefrom, are but a fraction of what would occur if the fermentation were induced by pure cultures of Leuconostoc species and protected from the competitive activities of the various groups of microorganisms invariably present in mill extracted juices and particularly from canes that have been ruptured by severe freezes. Under ideal conditions of temperatures and nutrient enrichments, a 15% sucrose solution can be completely solidified by a pure fermentation induced by Leuconostoc dextranicus in less than 48 hours, while the spontaneous fermentation of juices from its "epiphytic" flora is never anywhere nearly so effective, as regards the conversion of sucrose into dextran. The typical, as well as the most destructive, viscous fermentations that occur in mill juices from frozen cane are the resultant of a mixed microbial flora with yeast, mesophilic bacteria and mold fungi, all competing with L. mesenteroides for the sucrose present and the rapid lowering of the pH levels of the juice as a resultant of the activities of acid-forming bacteria and mold fungi render the substrate less and less suitable for dextran formation. To mill operators who have worked with frozen cane, the above may seem an undue minimization of the evils of the destructiveness of this baneful and dreaded scourge of sugar manufacture from freeze damaged cane. On the other hand, it emphasizes the immeasurable losses and the insurmountable obstacles to juice clarification that even a small amount of dextran in juices can cause. Like many other spontaneous fermentations in industry, the viscous fermentation accelerates with rapid momentum, for the causative agents become more and more vigorous as they are successively brought into contact with fresh substrate and as a

result they become far more efficient in producing dextran, until finally evidences of their activities may be seen all through the factory, wherever juices or dilute sucrose liquors are present. The species of L. mesenteroides then becomes so universally distributed throughout the factory that juice samples left exposed to the air immediately undergo this fermentation, even when the juice itself is derived from sound, undamaged cane.

Notwithstanding the unaminity of opinion, as regards the invariable sequence between frozen cane and clarification difficulties resulting from dextran formation in the juices extracted from them, there is still a broad hiatus in our knowledge of the causes for it. It is obvious that stalks of sugar cane, which have been ruptured or split by severe freezes, are exposed to all of the microbial contaminants that constitute the "epiphytic" flora of cane, but this does not fully explain the predisposition of juices from canes damaged in this way to viscous fermentations, nor their susceptibility to it. Canes damaged or killed by fire, or injured mechanically, so as to expose their interior to contamination from without, do not behave in this characteristic fashion, although their juices may present difficulties in clarification and processing. Nor does the juice from canes riddled with punctures, resulting from excessive infection by the cane borer, exhibit a special predisposition to viscous fermentations. The factors that may explain the characteristic behavior of juice from frozen cane and form the pattern for its specific type of deterioration, are as follows:

1. The low temperature that prevails at such times
2. The lowering of the protective action of the plant enzymes as a result of the freezing temperature

3. Changes in the form and the amounts of nitrogen in the juice as a result of the freeze.

Of the first of these factors, the writer (26) observed that cane juices held at a temperature of 55 degrees F. were more addicted to viscous fermentations than where they were exposed to higher temperature levels, which are more favorable for the growth of yeast and mesophilic bacteria. While the optimum temperature for dextran production is approximately 20° C., the possible reason for the attainment of higher viscosities in the lower, or psychrophilic, level of 12.5° C. is that at this temperature the rate of multiplication of competitive microorganisms is far lower, hence the field is more restricted in favor of the Leuconostoc species.

The second factor of assumed lower protective enzymic action of juices, from frozen cane, as compared with those from undamaged stalks, has not yet been fully investigated, although the writer has observed that raw juice boiled and either exposed to the air or inoculated with viscous juices do not consistently follow the dextran fermentation pattern, but in fact more frequently undergo alcoholic and acetic fermentation.

It is probable that there is some change in the form of the amount of the nitrogenous constituents of the juice, resulting from the freezing cane, that is the greatest factor in the addiction of such juices to dextran fermentation. Alford and McCleskey (27), as well as Hucker and Pedersen (28), have shown that Tryptone greatly accelerates the growth of all species of Leuconostoc and is markedly promotive of their growth and activities. We have already referred to the work of Iwata (29), who showed that the nitrogen in damaged canes was

greater than in sound ones of the same variety and the same stages of growth, but his observations referred to damages from cane borers and other insects, rather than from freezes. However, until chemical investigations have fully established the occurrence of such changes in the nitrogenous constituents of cane juices, as a result of freezes, we will have to accept this hypothesis with reservations.

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Chapter IV

FERMENTATION OF DIFFUSION JUICES--SUGAR BEETS

In beet sugar manufacture, opportunities for the fermentation of the juices extracted by diffusion are far less frequent than in the cane sugar manufacturing procedure and there are no typical deteriorative changes that occur under normal conditions, since the juice is extracted at temperatures between 75 and 85° C., at which temperature only the thermophilic bacteria could be active and these only at their maximum range of temperature adaptations. Another factor which also differs from cane manufacture is that the beets are washed before they are sliced and as a result the "epiphytic" flora and the adhering soil particles are removed before the raw material is introduced into the factory. We have already referred, however, to the investigations by Schone on the microbial content of the cossettes from freshly sliced beets and in several cases these counts attained levels of over a million bacteria per c.c. Even so, in spite of this high microbial population level of the raw material, the prompt exposure of the cossettes to temperatures above stated would preclude any, except a possible very limited, microbial development.

There is one type of fermentation, however, which is now very rarely experienced, but in early days was not infrequent, and that is the production of explosive gases in the diffusers, due to the formation of hydrogen.

Chevron (1), who investigated this phenomena, attributed the action of organic acids upon the

iron walls of the diffusers and the formation of explosive mixtures of gas. Except for the high temperature in such cases, one would be likely to assume that in these explosions from gas, resulting from fermentation, the butyl or butyric organisms were the causative agents. Strangely enough, however, Thaysen and Galloway (2), referring to Schone's investigations, bring out the interesting fact of cases recorded in which butyric acid bacteria had been isolated from fermented raw beet sugar. It is far more likely that the explanation for these gas explosions in the diffusers is that for some reason the temperatures had fallen far below the normal operating temperatures and spores of the butylic acid had an opportunity to develop and to initiate fermentation, by producing mixtures of hydrogen and CO2.

Although the viscous fermentation species of bacteria, such as Leuconostoc mesenteroides and Clostridium gelatinosum, are constantly present in beet juices, the opportunity rarely, if ever, occurs which permits them to cause any trouble and the temperature of the diffusion batteries is almost at a level at which the former organism is rendered inactive, although the second can, of course, easily withstand temperatures far higher.

It is very probable that the explanation for fermentation and the formation of explosive mixtures of gases in the diffusers in the early days and the complete absence of such incidents, during recent years, is that the temperature of diffusion water was formerly much lower than at present and Thaysen and Galloway state that in the early days of beet sugar manufacture the temperatures at which cossettes were extracted often fell below 60° C. and that in many cases the temperatures in the diffusion batteries were not above 40° C. Wherever such conditions existed, it could be well understood that butyric and butylic bacteria would

find it a most favorable condition for their action

Upon leaving the diffusion batteries, the extracted juice is immediately defecated by the addition of lime to increase its pH value from between 4.8 and 5.2 to 7.2 and 8.5 and this clarification, together with the fact that the juices boil at this stage, serves to practically sterilize the product.

In view of this procedure, the handling of sugar beets presents no bacteriological problem comparable to that of sugar manufacture from cane, as regards the deterioration of extracted juice and the development of microorganisms.

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Chapter V

APPRAISAL OF MICROORGANISMS CANE JUICES AND BEET DIFFUSION LIQUORS

Bacteriological analyses of mill juices in cane sugar factories and diffusion liquors in beet factories have never become a routine procedure, for only on very infrequent occasions is it necessary to appraise the agencies responsible for undetermined sucrose losses and these are seldom suspected as resulting from microbial activities in the raw juices. And in those fortunately rare occurrences, where cane has been visibly effected by freezes, the behavior of the juice, as regards erroneous polarizations and difficult filtration, constitutes adequate presumption that dextran fermentation has occurred and is sufficiently valid to dispense with any qualitative bacteriological analysis. On the other hand, one can scarcely doubt that continuous bacteriological control of raw cane sugar factory operations would reveal definite and consistent losses from microbial activities, that is provided that the chemical methods employed for their detection are sufficiently sensitive to serve that purpose. Were such procedures employed, there is but little doubt that comparisons of the acidities in crusher and last mill juice (expressed as acidity per 100 Brix) would be found to run parallel with the increases in microbial content of the juice at these two stages of mill extraction (cf McCleery, also Spencer-Meade Sugar Handbook). Continuous bacteriological control, correlated with sensitive chemical analyses of crusher and mill juices, is indispensable in the maintenance of adequate mill sanitation, which becomes more and

more defective as the tendency to multiply milling units, in order to obtain the highest possible mill extraction, becomes more universal. The problem is still further aggravated by the increasing dependence of the cane sugar industry upon mechanical harvesters, which decrease labor costs, but at the expense of increasing percentages of trash brought to the mills. In anticipation of the none too distant day when the importance of bacteriological control of raw sugar factories will be a general practice, as well as the not too infrequent need for it as a check on raw juice deterioration, a list of methods and procedures are given in the following pages.

QUANTITATIVE METHODS FOR THE BACTERIOLOGICAL ANALYSIS OF RAW JUICES

1. Collection of Samples

Samples should be collected in wide-mouth, sterile bottles, or pint fruit jars, which should be provided with a fine mesh-wire strainer or a section of flannel or cheese cloth. The strainers can be sterilized in position and the top of the jars loosely fitted to prevent contamination. It is important to strain the bagacillio and trash from the sample, as these mechanical contaminants usually carry a concentration of microorganisms far greater than in the juice proper. Samples of crusher juices and mixed juices should be taken, so as to arrive at the relative degree of microbial contamination at both stages of milling. This difference, when too preponderantly large in the mixed juice, affords an index of the state of sanitation of the milling units.

The strained samples should be analyzed as promptly as possible, or if this is not practicable, they should be kept well iced or preferably

packed in dry ice, to retard microbial growth, which is very rapid in raw juice.

2. Dilutions for Plating

It may be assumed that the average mill juice will contain mesophilic bacteria and yeast in the ranges of from one hundred thousand to several million viable microorganisms per Cc. Millstein, Tobin and McCleskey (1) reported a low of 5,000,000 and a high of 570,000,000 in the juices that they investigated in Louisiana factories. Earlier results by the writer and others were somewhat lower (2), but the difference is probably the result of an improvement in the substrate employed for the assay by the later workers, rather than to any change for the worse in mill sanitation.

Dilutions of from 1:100,000 to 10,000,000 usually provide a suitable range for the average mill juice and these dilutions may be obtained as follows:

> One Cc. of the juice is withdrawn from the sample jar, after the latter is thoroughly mixed by shaking, and transferred to a 2-liter Erlenmeyer flask, containing 1 liter of sterile water. From the latter, another one Cc. portion is transferred to a dilution flask containing 100 Cc's of sterile water. From this latter water blank, one cc. portions are withdrawn and introduced into sterile petri dishes, of which at least three should be employed to obtain an average count. On these plates, each colony that develops represents one hundred thousand in the original sample.
>
> From the 1:100,000 dilution water blank, a 10 cc portion is transferred by

a sterile 10 cc. pipette to a 100 cc. water blank, from which one cc. portions are transferred to three plates, as above described. Each colony developing on these plates represents 1,000,000 microorganisms in the original sample and the count on the 3 plates are averaged as before.

To obtain a dilution of one to ten million, ten cc's of the second 100 cc. water blank is transferred to a third 100 cc. sterile dilution blank and 1 cc. portions plated out as before, so each colony on these plates represents 10,000,000 microorganisms in the original juice.

3. The Substrate or Culture Medium for the Bacteriological Assay of Raw Juices

For a general purpose solid substrate or culture medium for raw cane juice analysis, the medium used by Millstein, Tobin and McCleskey seems to admit of higher counts than others and is to be recommended for this purpose. Its composition is as follows:

Raw Juice Tryptone Agar

Tryptone	10 gr.	Agar	20 g.
NaCl	1 gr.	Filtered cane juice*	1000

*Raw juice heated and filtered through cloth

The writer (3), in an investigation of the comparative values of five different substrates for the appraisal of mesophilic bacteria, in raw cane juices, obtained higher values with nutrient agar than with others containing cane juice, which is suggestive of the preponderance of irrelevant species of bacteria in the samples that were then

investigated. The levels of bacterial population revealed in that study were far below those that were later found by the above authors to be typical of raw cane juices. The difference may probably be attributable to the use of tryptone in the raw juice agar employed by them, as contrasted to the peptone used in ours.

Composition of Owen's Raw Juice Agar

Fresh Raw Cane Juice Heated and Filtered through Cotton and Solidified with 2% Agar

For the estimation of mold fungi in raw cane juices, the substrate proposed by the Kopeloffs (4) and employed extensively by them, in their study of the occurrence of these microorganisms in sugar house products, was as follows:

Cane sugar	50.00 grams	$FeSO_4$	0.01 gram
Peptone	5.00 grams	NH_4NO_3	1.00 gram
K_2HPO_4	1.00 grams	Agar agar	20.00 grams
KCL	0.50 grams	Water	1000.00 cc.
$MgSO_4$	0.25 gram		

APPRAISAL OF VIABLE YEAST IN RAW CANE JUICES

It is seldom that occasion arises to determine the concentration of viable yeast in cane juices; however, there are frequent times when the search for highly efficient yeast for use in producing rum leads to a search of raw juices for strains and species of highly desirable yeast for that purpose. Sugar cane juices and molasses are both a veritable storehouse for unusual and desirable ester forming yeasts, for use in rum distilleries, and for this purpose the following substrate will be found most satisfactory:

Blackstrap Molasses
diluted to 10 Brix ..... 1 liter)
Ammonium Sulphate ..... 1 gram) Agar Agar
Ammonium Phosphate ..... 1 gram) 20 grams

The juice can be either diluted from 1:100 or 1:1000 and plated out on this substrate or the plates can be poured and allowed to harden and the surface streaked with a platinum loop of the juice after the loop has been sterilized by flaming. On this medium, the yeasts grow much better than bacteria and the yeast colonies can be identified by their moist and raised appearance, as well as by their typical very white color.

THE QUANTITATIVE DETERMINATION OF THE BACTERIA IN RAW CANE JUICES BY THE METHYLENE BLUE REDUCTION METHOD

Some years ago, the writer attempted to apply the Methylene Blue reduction method, now commonly used in the determination of the bacterial concentration in raw milk, to determine the number of microorganisms in raw sugars. It was thought that this might be a very simple and practical method of appraising the microbial concentrations in sugar, but the results were not satisfactory, owing, of course, to the low levels of the bacterial population of sugars, as compared with that of milk. However, there is but little doubt that when the occasion warrants it could be applied very easily to raw cane juices, where the levels of bacterial concentrations are comparable to those of milk. The procedure for use of this method in the Dairy Industry is as follows:

PIPETTES, BURETTES AND DIPPER *

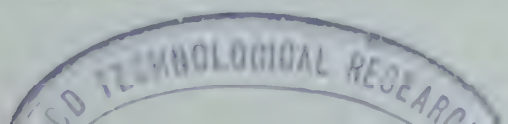
Measure the quality of juice to be tested with either a sterile straight-sided one-mark pipette, graduated to deliver 10 cc. or a dipper which will deliver 10 cc. Subject the dipper to practical sterilization, as hereinafter described. Use a graduated burette, either mounted or unmounted, or a one-mark pipette graduated to deliver 1 cc. to measure the methylene blue solution into the milk.

CULTURE TUBES

Provide culture tubes without lip, length 150 mm. and diameter 12-15 mm. Close the tubes with rubber stoppers to protect the contents from contamination during the usual manipulations in collection and incubation. If special tubes with similar dimensions and graduated to contain 10 cc. are used, fill the tubes to the mark with any convenient instrument which is sterile or has been subjected to practical sterilization between samples.

WATER BATHS AND TUBE RACKS

Incubate the tubes of juice at 37° C., tolerance = 0.5° C., preferably in a water bath of sufficient capacity to warm the samples to 37° C. within 5 minutes after placing therein. An air incubator thermostatically controlled at 37° C. = 0.5° C. may be used optionally, providing, (a) the samples are warmed to 37° C. before placing



therein; (b) observations are made without lowering the temperature of the juice; and (c) the samples are completely protected from light rays including those from the heat source and the pilot light. Do not put the tubes in the water bath in such large numbers at any one time as to delay the warming period more than 5 minutes. Provide a thermostatically controlled heat source for the water bath to insure uniformity of temperatures. A hand controlled flame may be operated within the tolerance for heating the bath, but in practice it is seldom done because of inconveniences and labor cost.

Direct sunlight and even strong electric light are known to catalyze the reduction of methylene blue in juice. Protect the tubes from strong light if it is necessary to incubate them in a window beneath a skylight, in the direct path of rays from the sun, or within 6 ft. of an electric light. All-glass or glass-front water baths should be painted to prevent transmission of light through the glass. Do not use electric light bulbs immersed in the water unless the tubes are completely protected from the rays of light.

Provide rust-resistant sheet metal or wire racks for arrangement of the test tubes in single or double rows and so constructed that each tube is held in a vertical position in the water bath or incubator to insure at all times against disturbance of the contents of the tubes.

METHYLENE BLUE TABLETS AND SOLUTION

The early European technic resulted in a concentration of approximately 1 part of methylene blue to 300,000 parts of juice, while the American and later European procedures have resulted in concentrations considerably weaker than this. The proper concentration is the weakest which will give a sharp end point of reduction. A concentration of 1 part of dye to 300,000 parts of juice gives satisfactory results, when the procedure outlined below is followed. This is a stronger concentration than was obtained with the former American methylene blue chloride tablets and will lengthen somewhat the average reduction of juice. This increased reduction time is due to increased concentration of the dye and not to substitution of methylene blue thiocyanate for methylene blue chloride.

Use only the methylene blue thiocyanate tablets which have been certified by the Commission on Standardization of Biological Stains to be suitable for the methylene blue reduction method. Dissolve one tablet in 200 cc. of sterile or freshly boiled distilled water, observing that the volume of the solution, when at room temperature, is 200 cc. The present methylene blue thiocyanate tablets are harder, less easily chipped or pulverized, and require more time to dissolve than the originally used methylene blue chloride tablets. Solution is complete when one tablet is allowed to stand in 200 cc. of water over night.

Always protect the aqueous solution of the dye from light to prevent its rapid

deterioration. Store the dye in amber glass bottles in the dark. In practice, a fresh solution is usually prepared weekly to avoid the problem of deterioration.

MAKING THE TEST

Use glassware which is scrupulously clean and free from etching to prevent precipitation of the methylene blue salts. Cleaning solutions may be used. Sterilize the containers for the dye solution, the pipettes, test tubes and rubber stoppers, if possible. If impossible, boil the glassware and stoppers immediately before use and allow the retained heat to dry the equipment. Avoid recontamination of that portion of the sterilized stopper which is inserted into the test tube.

With a burette or a 1 cc. pipette, add 1 cc. of the methylene blue thiocyanate solution to the tube, either a short time before or after the juice has been placed therein. Identify each tube legibly and indelibly immediately before or after the addition of the sample with the producer's number or in some other suitable manner. Stopper the tubes immediately and place them in the water bath at 37° C. After 5 minutes have elapsed, invert the tubes once, after which avoid agitation which might disturb the surface layer.

The methylene blue reduction time is the interval between the placing of the tubes in the 37° C. water bath and the disappearance of the blue color from the milk which regains its normal original color appearance. Use

different time intervals to indicate different grades of milk.

*(Standard Methods for the Examination of Dairy Products. 7th edition, 1939).

The writer has found in his preliminary experiments that this procedure can be applied without any extensive modification to the estimation of bacteria in raw juices.

This method would be of great advantage in correlating the acidities of juice with bacterial population and would be a further check of the acidity criteria as a basis for rejecting cane which is presumed to have been damaged by freezes. This method is so simple and so rapid in comparison with the usual bacteriological assay that it could easily be applied to raw juices in any sugar factory which has even the most modest of laboratory equipment. Any control factory laboratory could easily introduce it as a regular practice and it would provide a very practical means for the maintenance of sanitation in mills, since it would show progressive degrees of contamination made in or around the mill, to which the raw juice is exposed.

QUALITATIVE METHODS FOR THE DETECTION OF BACTERIAL SPECIES

There are times when a qualitative bacteriological analysis of raw juices becomes of great importance and this is especially the case when canes are being milled which are suspected of having been damaged by freezes and the juices from them are increasingly difficult to clarify. In the incipient stages of these difficulties, it is often of primary importance to determine if dextran-forming bacterial species occur in numbers

which indicate the initial formation of gums. While the appraisal of the quality of the juice from a manufacturing standpoint is usually dete mined by its sucrose values and its acidities, the prevelance of detectable numbers of Leuconc mesenteroides, or other species of dextran-form bacteria, might be even more valid criteria of juice and the nature of the difficulties that i will give, as regards clarification and sucrose recoveries. For this purpose, the Alford and McCleskey (5) substrate will be found to be mos efficient. The above investigators found that sucrose tryptone agar and glucose yeast extract agar were about equally suitable for the purpos of isolation of Leuconostoc mesenteroides, but sucrose tryptone agar, being a purer medium, ad mits of a clearer differentiate of these specie from the others that may grow on these media.

Glucose	1 gram	Beef extract	3 gra
Yeast Extract	1 gram	Agar	15 gra
Tryptone	10 grams	Distilled water	1 li

McCalip and Hall (6) employed a sucrose ge tin substrate in their investigation of L. mesenteroides in juices and the formula which t employed is as follows:

Sucrose	50 grams
Gelatin	120 grams
Water	1 liter
pH adjusted	6.8 to 7.0

Note: This substrate was employed for the detection of Leuconostoc mesenteroid on stalks of frozen cane. 10 grams of each section were weighed into 90 grams of sterile water for plating and this water extract from the cane may have added some essential growth factors to the medium.

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Chapter VI

PROGRESSIVE DECIMATION OF MICROORGANISMS INCIDENT TO THE MANUFACTURING PROCESS

Every step in the clarification of juices or sugar liquors, incident to the manufacture of raw or refined sugars, contributes to the sterility of the product treated and irrespective of what clarification system is used the results are very much the same in that only traces of the original flora survive. As we have previously pointed out, the mere filtration of unheated juices serves to greatly reduce the numbers of microorganisms in the filtrate, as compared with the unfiltered juice and it may be assumed that filtration preceded by flocculation at boiling temperatures will not only kill and remove all viable vegetative cells, but a large part of the bacterial spores as well. It might be well to outline the industrial practices in the clarification of raw juices in raw sugar factories and raw sugar liquors in refineries and diffusion juices from beets, in order to show how effective these sterilization procedures are in theory and to more clearly perceive and explain the presence of any chance survivors of these processes. In raw sugar manufacture the general clarification procedure is as follows:

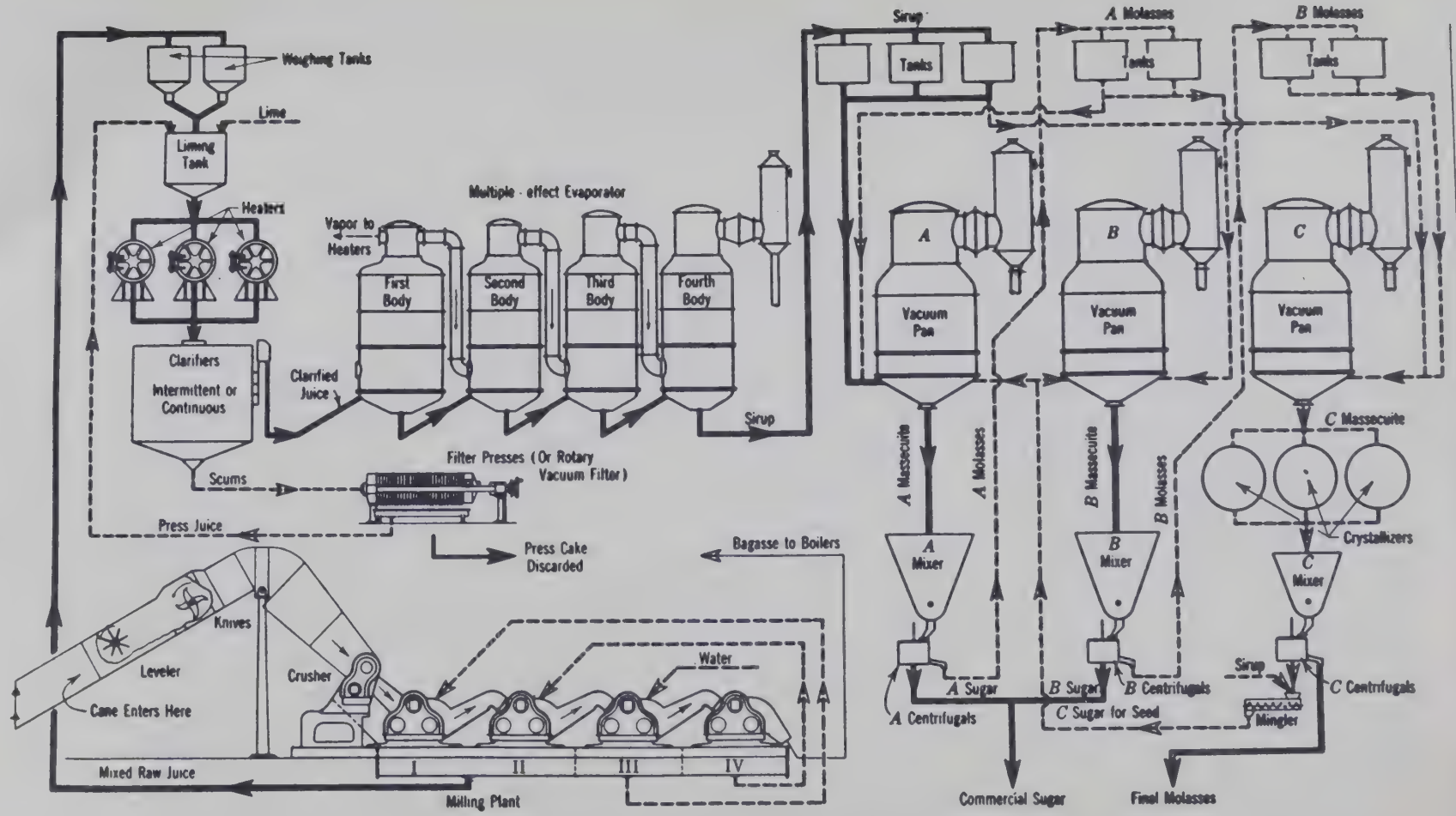
1. Liming to a pH of 7.00, heating to a temperature of 194-238° F.
2. Settling
3. Pumping through juice heaters to the effects
4. Concentrating the syrups in vacuum pans
5. Separation of the crystals in centrifugals
6. Bagging the sugar direct from the centrifugals.

There are many modifications of the standard procedure for raw juice clarification, the most important of which are:

A. Fractional Liming
B. Fractional liming and double heating
C. Addition of lime as saccharate
D. Addition of superphosphates to limed juices
E. The carbonation process (Chiefly for production of direct consumption sugars)
F. The Sulfitation process, using SO_2 and lime (Chiefly for the production of white sugars)

All of the procedures and inovations in the clarification of raw juices and raw sugar liquors have been designed either to give more flocculent and more rapidly settling precipitates or to effect the greatest possible removal of the most objectionable of the impurities in the juices, such as gums and other colloids. The more effective the elimination of these impurities and the more rapidly settling nature of the precipitate formed from the combination of lime and heat or lime and superphosphates, or lime and sulphur dioxide, the greater will be the removal of microorganisms. In this connection, the controversial question as to whether cold or hot liming is the more effective procedure for the removal of gums and colloids and the assumption that better elimination is obtained from the addition of the lime to the heated juice, might be approved entirely from a microbiological standpoint, owing to the fact that heating the slightly acid juice (pH 5.10-5.40) would be more destructive of microorganisms than an exposure to the same temperatures, after liming, to neutrality or slightly above.

It is the writers opinion, based upon a rather extensive investigation of the microorganisms



Flow Diagram of Raw Sugar Factory.

Courtesy of John Wiley & Sons, New York, Publishers of Spencer-Meade Cane Sugar Handbook, Eighth Edition.

occurring in raw and refined sugars, that the phosphoric clarification tends to give lower levels of bacterial population in sugars than is obtained by the straight lime process and that in refineries where this clarificant is employed the thermophilic count of the sugars is correspondingly reduced. However, the importance of securing maximum obtainable purity increases of the treated juices so greatly outweighs considerations of the elimination of microorganisms that few instances are recorded in the literature of proposals that standard clarification procedures in raw sugar factories should be modified to serve bacteriological objectives. However, Geerligs (1) recommended that heating the juice before liming serves to reduce the development of Leuconostoc mesenteroides and that this procedure should be practiced where infection of juices with this species is suspected. Incidentally, this procedure has also been confirmed as meritorious upon the basis of the reduced solubilities of crude gums in acid medium and their greater tendency to flocculate by heat at a pH below 7.0 than in the cold limed juices at pH levels nearer neutrality or on the alkaline side.

In the various modifications of the conventional clarification methods and practices incident to the production of raw sugars, it was but natural that attention should have been directed to the segregation of the richer juices from crushers and first mills and treating them separately, so as to avoid the difficulties incident to blending in the initial stages of manufacture, the rich and the poorest juices extracted from the cane. In this procedure the mud from the juices (crusher and first mill) are sent to the primary defecator and the muds from the primary defecator are mixed with juices from the second mills and go to the second defecator and the secondary clarified juices are sent to the primary defecator. With a view of

eliminating the costly and at best untidy filter press stations in raw sugar factories, various procedures have been elaborated, designed to return the muds to the last rollers of the milling system, thus utilizing the bagasse as a filtering medium and disposing of the muds with the bagasse as the latter is diverted to the furnaces of the boilers. The Petree Process was designed on this basis and was the initial step towards the compound method of clarification as used today. In the Petree system, the mud waters were diluted with the juices from the last mill and this diluted mud was then pumped to the bagasses behind one of the preceding mills. The savings, incident to the elimination of the filter press, were more than offset by losses in sucrose recoveries and by reduced mill extraction. It is not unlikely that no inconsiderable part of the losses in sucrose was the result of microbial activities resulting from the return of this highly contaminated mud to the ideal substrate offered by freshly extracted juices. While this system was abandoned after a few abortive trials, it was the precursor of the conventional types of compound clarification in use today. Where there are some mills which even now use the return mud system, it is in a greatly modified form from that used in the original Petree procedure.

Liming at the mills has also been practiced both as a means of protecting the juice from the action of microorganisms and also as an accelerant of clarification procedures that follow its extraction, but these results were not satisfactory, owing to the adverse effect of the treatment upon the injurious constituents of the cane fiber, which were brought into the solution by the lime. As for its protection against microbial activities in the juice, we have already referred to the investigations of Carpenter and Bomonti and of McCleery (cf Chapter II).

From microbiological considerations, we have to include in the review of various clarification practices some appraisal of the constituents removed in the process, particularly where they may be of a nature that might either serve as nutrients for microbial growth or as stimulants of microbial activities - that is to say that whether the clarified juices or liquors would support microbial activities as well at a given level of microbial population as would the original substrate. Such an appraisal would include the following constituents of the unclarified material:

1. Its nitrogen content
2. Its ash constituents (particularly phosphates)
3. Its colloids
4. Mechanically suspended matter.

As regards nitrogen elimination in the clarification of juices, Davies (2) has contributed some comprehensive data and we are indebted to him for the following data (given on page 64) from his publication.

We have here in the following table a comparison of the relative efficiencies of two procedures of clarification on the juices from two different varieties of cane. The elimination of protein varies from 54 to 83 on one variety and from 84 to 94 in the other. The elimination of colloids varies from 18 to 32 percent on one juice and from 22 to 36 on the other, so it may be concluded that any effective system of clarification, as measured by juice clarity, will remove most of the nitrogen originally present in the raw juice. In fact, Fort (3) has claimed, from his extensive investigation of cane juice clarification, that the two constituents chiefly involved in the increases in purity resulting from clarification are the

FACTORY COMPARISON OF THE COLD LIMING AND OF THE FRACTIONAL LIMING AND DOUBLE HEATING PROCESS

I. C. T. A.* EXPERIMENTAL FACTORY

Variety	C. L. Process		F. L. & D. H. Process	
	B.H. 10(12)	P.O.J. 2878	B.H. 10(12)	P.O.J. 2878
Gravity Purity rise (M.J. to Cl.J)	1.80	0.14	2.38	1.86
Colloid Elimination (Dye Value) %	32	18	36	22
Protein Elimination %	83	54	94	84
Mud Volume %	10.7	15.8**	5.0	10.7
Time of settling hours	.75	1-2.5	.75	.75
Liming saving %			33.7	39.5

*Imperial College of Tropical Agriculture, Trinidad

**This figure does not include one experiment in which the juice showed no sign of settling after 2.5 hours and should therefore have been recorded as 100% mud. The corresponding F.L. and D.H. figure includes this juice treated by that process.

protein and the phosphate constituents of the juice. It might be said, also, that these two constituents exercise a most important role in the susceptibility of a juice to clarification procedures and Salinas (4) states that for good clarification, a juice should contain not less than 0.10 per cent of albumin and 0.030 percent of P_2O_5.

The elimination of phosphates by clarification may be observed in the following table from the published report by Davies:

P_2O_5 CONTENT AND OF CLARIFIED JUICE, EXPRESSED AS P_2O_5 PERCENT TOTAL SOLIDS

Variety	B. 7					
	Raw	Clarified	Raw	Clarified	Raw	Clarified
	0.088	0.029	0.091	0.029	0.184	[illegible]
	0.062	0.027	0.106	0.024	0.170	[illegible]
	0.055	[illegible]	[illegible]	[illegible]	0.089	[illegible]
Mean		[illegible]		[illegible]		[illegible]

It is evident from the foregoing data on the effect upon the chemical and physical composition of cane juice by the conventional clarification used in raw sugar manufacture that the clarified product is far lower in nutrients and microbial growth and in addition the removal of colloidal material greatly decreases the quality of the product when viewed in the light of a substrate for the development of microorganisms. In addition, it is very likely, although definite data is not available, that the growth-promoting factors, such as vitamins, are largely eliminated in the clarification process. It has been the writer's experience in the investigation of the rate of growth of mixed microbial cells in clarified juices that the growth curve is far less steep than is true of raw juices having the same levels of viable cell concentration. The assumptions are, therefore, even in the rare instances where clarified juices may be exposed to normal temperatures for some hours, that the rate of deterioration would be far lower than would be the case of the unclarified product and this has a great bearing, naturally, upon the values obtained for the clarified juice in raw sugar factories. Referring again to the various published data on the decrease of

microorganisms during raw sugar manufacture, it is not surprising to note that the clarified juice is usually almost sterile and probably the only viable cells that it contains are in the form of bacterial spores, which are very resistant to heat. As regards mesophilic microorganisms, that is those which grow at the ordinary temperatures, the increase in microorganisms throughout the various evaporating stages is probably explained by the accumulation of viable cells in the distributing lines rather than the results of any actual microbial growth in the process.

THE PERSISTENCE OF THERMOPHILIC BACTERIA THROUGHOUT THE VARIOUS STAGES OF RAW SUGAR MANUFACTURE

In considering the survival rate of thermophilic bacteria during raw sugar manufacture, we must take into account the fact that these species are sporogenous and their spores are highly resistant and hence are capable of surviving throughout all of the stages of sugar manufacture and their elimination is entirely one of removal by filtration, rather than being killed by heat. On the other hand, we are warranted in assuming that these species are exclusively in the spore form, as they occur in the raw juices or in the contamination in the factory. The grounds for this assumption are that the temperature limits for their growth are beyond the levels at which raw juice is extracted and hence it is only when the juice is heated and cooled to ranges between approximately 40 to 65° C. that any growth can be expected. Referring again to Millstein, Tobin and McCleskey (5), whose figures are shown below, the levels of concentration of thermophilic bacteria continue at rather high levels throughout the entire process.

Sample	Low	High
Raw juice	14	170
Seip effluent	0	4
Dorr effluent	0	8
Press juice	3,700	205,000
Evaporator	200	15,500
Storage tank	16,100	38,500
Crystallizer	350	15,000
Massecuite	1,700	17,100
Raw sugar	100	2,200
Molasses	1,200	16,500

Under certain conditions, but upon rather infrequent occasions, the concentrations of viable spores of these species in clarified juices and in syrups from the effects lead one to suspect a development, rather than an accumulation, of acquired contaminating material is responsible for this condition. Yet the ability of these species to develop in these products, even when the temperature levels coincide with the optimum conditions for growth of thermophilic bacteria, still lacks valid proof and other than the work of Carpenter and Bomonti (6) and other sugar technologists of Hawaii, there is no data to support this theory and their data on the deterioration of juices at high temperatures did not include actual bacteriological analyses. Nor does the behavior of the thermophilic species responsible for the deterioration of certain canned food in which sugars are used, particularly sweet corn, suggest their ability to induce changes in clarified juices or syrups and the writer has never been able to

detect any material change in such products inoculated with these bacteria and stored at optimum temperature levels for the development of thermophilic species. Contrary to the substrate requirements for the mesophilic bacteria normally found in sugars and cane juices, the nutrients and growth factors required to develop colonies of the sugar thermophilic species are quite complex and it is assumed that the nutrient requirements of the latter are both exacting and intricate. The possibility should not be overlooked, of course, of the existence of other thermophilic species, different both as to their adaptability to simple sugar solutions and as to their capacity to utilize it in their metabolic activities. It is not at all impossible that the species which are revealed in the conventional thermophilic bacteriological analyses applied to refined sugars, to determine their suitableness for canning purposes, do not include others that may be responsible for the actual sugar losses in raw sugar manufacture and raw sugar refining.

One of the inherent difficulties in measuring or even in detecting small losses in sucrose, either in the plant or in the laboratory, is that at high temperatures and pH levels of 7.0, and above, both sucrose and levulose may be destroyed at equal rates and as Meade (7) points out this may confuse the interpretation of the glucose ratios and lead to the belief that no sucrose has been lost, since the validity of this relationship is based upon the assumption that no reducing sugars have been decomposed.

The possibility of losses in levulose, even at a temperature of 55° C., the optimum temperature for the development of thermophilic bacteria, is a real one when the product is exposed for a period of 48 hours, the usual incubation time for

thermophilic appraisal in sugars, and hence the real effect of the growth of these bacteria in sucrose solution containing levulose may easily be overlooked.

THE POTENTIAL ZONES OF DEVELOPMENT OR ACCUMULATION OF THERMOPHILIC BACTERIA IN RAW SUGAR FACTORIES

While but little attention has been paid to the survival of thermophilic bacteria in raw sugar manufacture, since no standards are applied to these products with reference to their microbial content, as are applied to refined sugars used in canning, sufficient data has been acquired from the extensive investigations of the occurrence of these species in sugar refineries to serve to explain their constant presence at all stages in raw sugar manufacture. From the data of Millstein, Tobin and McCleskey (8), shown on the previous pages, it will be seen that the raw juice contains comparatively few of these bacteria and the effluents from the defecators are practically free of them. On the other hand, the filter press juice contains a large number of thermophilic bacteria and their numbers may also be high in syrup storage tanks. Calton (9) observed a similar concentration of these bacteria in the pan storage tanks of sugar factories and in the fifth body steam chest of multiple effects in a beet sugar factory. He also found that the first filtrate from freshly coated plates of filter presses contained high concentrations of these microorganisms and this high level continued for the first hour of operation. In the observation that syrup and pan-storage tanks contained a relatively large number of thermophilic bacteria, most of the observers have found that this condition is associated with the presence of a surface deposit on the tank walls,

which would indicate that the high cell concentration of these species is the result of the flotation of the accumulated contaminants and their concentration at the surface of the stored material. However, it is possible that it represents a growth at zones at the surface, where the cooling of the syrups is more rapid than in the liquid proper. Similarly, Calton has observed, also, that in the beet factories which he investigated there was an increase in the concentration of thermophilic bacteria between the filter presses and the pumps and in this observation actual growth, rather than deposition of contaminating material, is indicated.

While it has been claimed by some investigators, particularly Cameron and Bigelow (10) and Cameron and Williams (11), that clear colorless char filter liquor will usually contain not more than one or two thermophilic spores per 6 grams of sugar, while the darker liquors from the final run of a filter give, as a rule, 100 or more per gram of sugar, the writer has not always found that the efficiency of filtration, as measured by color removal and the purity rises can be correlated with the efficiency of the elimination of thermophilic bacteria. It is apparent, of course, that the first clear liquors from a char filter will usually contain fewer microorganisms of all kinds than the low purity residual run-offs, but the refiner has no way of appraising the level of thermophilic bacteria in any filtrate from its excellence, as regards chemical and physical purity.

We may conclude, therefore, that the increase and decline and the rise and ebb of the levels of thermophilic bacterial population, during the course of raw sugar manufacture, is a resultant of adventitious contamination at undetermined frequencies or the intermittent failure of clarification in removing these microorganisms and

occasionally perhaps the result of the concentration of the accumulated contaminants at the surface of storage tanks. In this latter connection, it is possible that cane wax may be the flotation agent that concentrates these bacterial spores at the surfaces of stored syrups or molasses.

The writer's attention has been called on several occasions to reported instances of inordinately high undetermined losses in sucrose during char filtration of sugar liquors and in one instance an occasion was offered to subject the phenomenon to a thorough bacteriological investigation. All factors pointed to the char filtration station as the zone of sucrose destruction and chemical analyses showed conclusively that the sucrose drop occurred there; but in spite of all attempts to isolate from the filtered liquors or from the spent char, bacterial species capable of inverting sucrose at the temperatures prevailing in the filters, the results were entirely negative. Subsequent chemical investigations revealed that the sucrose losses were the result of improperly and inadequately burnt or revivified chars and upon the correction of this defect, further losses were avoided.

THE SURVIVAL OF MICROORGANISMS IN BEET SUGAR MANUFACTURE

The procedure followed in bringing the raw plant material into the factory in beet manufacture differs essentially from that of raw cane sugar plants and the extraction of the juice in the two cases is, as is well known, essentially different as the extraction of the juice from cane is by milling and that from beet by diffusion. From a microbiological standpoint all of the essential differences in the two processes contribute to a

lower microbial contamination of beet diffusion juices than is true of mill extracted cane juice. The differences that contribute to the lower level of contamination of beet juices are (1) that the beets are flumed into the washing machines with waste water, (2) they receive an additional mechanical cleaning while in transit to the washers and, hence, are freed from mechanical impurities, such as gravel or particles of soil, etc., (3) they are weighed in an automatic weigher and discharged into a hopper from which they are fed to the slicers and (4) from the slicers they are fed directly to the diffusion cells, where they are immediately brought into contact with diffusion water at a temperature of approximately 80-90° C. Since the beet slices are free from extraneous soil and plant material and are promptly exposed to temperatures above the level to which even thermophilic bacteria can adjust themselves, there is but little chance for any microbial growth during the extraction stage of beet sugar manufacture. While the cane milling systems offer an ever present source of contamination and present almost insuperable difficulties, as regards sanitation, the beet washers and diffusion systems are easily cleaned and seldom offer any problems, as regards the maintenance of sanitation. However, any exceptional condition resulting in improperly washed beets are more conducive to high thermophilic contamination in beet factories than is true in raw cane sugar plants, owing to the fact that in cane juice clarification the juice is more promptly exposed to temperatures more nearly approaching the zone of sterilization than is true of beet sugar manufacture, where the diffusion juice is never exposed to temperatures even near the atmospheric boiling point of water. In cane juice clarification, on the other hand, the clarification temperatures are always above 212° F. and the trend is towards pressure heating.

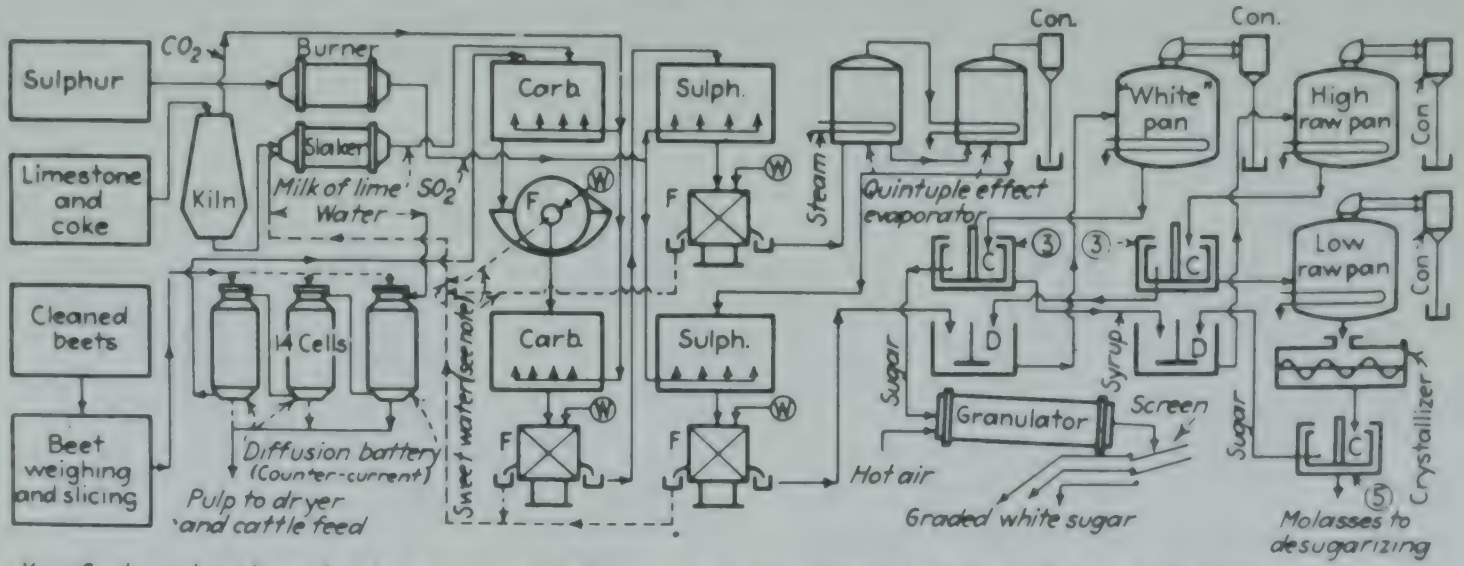
It will be apparent from the above description of the difference in the processing of cane and beet juices in the manufacture of sugar that the opportunities for the survival of resistant species of microorganisms, especially spore-forming bacteria, are much greater in beet sugar than in cane sugar factories. Hence, it would be expected that at certain times the number of viable spores of thermophilic bacteria might be quite large under these conditions and that at various intervals their level might be very high.

From the diffusion batteries the extracted juice is run into large tanks, where it is treated with quick-lime and milk of lime in the amount of 2.0 to 2.5% on the weight of the beets in terms of calcium oxide. Here the temperature of the juices varies from 70 to 80 degrees and sometimes as high as 85 degrees. The limed juice is then subjected to a double or triple carbonation process, during which process carbonic acid is forced into the limed juice through distributing pipes, and the lime is precipitated as a carbonate, or as salts of the acids of the juice. The flocculent precipitates of calcium carbonate carry down with them almost all of the suspended matter and presumably the large part of the microorganisms that occur there. The carbonation is carried to a point where the juice retains an alkalinity equivalent to about 1.0 to 1.5 grams of calcium oxide per liter, using phenolphthalein as an indicator. During the carbonation, the juice has a very pronounced tendency to foam and procedures have to be adopted to break this foam, either by the addition of grease or some surface depressant. This foaming tendency is conducive to the survival of microorganisms, owing to the fact that the foam rarely attains the temperature of the main body of the juice. The carbonated juice is then heated to near boiling and is put through the filter

presses from whence it flows to the second carbonation tanks. Sometimes it is necessary to add additional lime before the second carbonation and the temperature maintained during this operation is usually 100 degrees C. During this second carbonation, the reaction of the juice is brought near alkalinity. At times it is found that too much lime is added and the juice will tend to develop viscosity during evaporation and it may be difficult to work in the vacuum pans. In the liming process in beet factories the precipitates formed are very similar to those encountered in the clarification of cane juice, the salts of organic and inorganic acids, namely, phosphates and oxalates, and the albuminoid material coagulated and precipitated. The calcium carbonate usually precipitates much of the coloring matter, also, and the clarified juice is very light in color.

With reference to the comparatively low temperatures attained in the clarification of beet juices, it should be pointed out that the temperatures previously indicated apply only to where sound beets are being extracted, for where damaged beets or unsound beets are used even lower temperatures have to be employed, as the high temperatures may extract too much undesirable solids from the beet slices, hence where a large percentage of the beets being extracted are unsound much lower temperatures have to be used and the opportunities for the survival of microorganisms correspondingly increase. A flow sheet of the processing of sugar beets in the manufacture of sugar is given in the following chart:

BEET SUGAR



Key: Carb., carbonators; Sulph., sulphitors; F., filters; W, wash water; C., centrifugals; Con., condensers; D, dissolvers

Note: About half of all plants use sweet water from filter washing for slaking lime; others use fresh water

Farm land	0.6 acre	Coal (power and heating)	1.0 ton	To Produce
Sugar beets	8.0 tons	Sulphur	10 lb.	1 ton refined sugar
Limestone	0.5 ton	Water	90 tons	800 lb. molasses (50 per cent sucrose)
Coke	0.05 ton	Direct labor	16-20 man-hr.	800 lb. dried pulp

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THE SURVIVAL OF MICROORGANISMS IN SUGAR REFINING OPERATION

In contrast to either raw beet sugar or cane sugar manufacture, the operations incident to suga refining differ essentially, both as regards the number of microorganisms introduced into the proc- esses with the raw material and the conditions of viability of these species. As we have observed in the case of raw sugar manufacture, either cane juice or beet may have an "epiphytic" flora which consists largely of vegetative cells of the variou species of bacteria constituting this flora. At the time that the juice leaves the mills in the case of cane sugar manufacture, or at the time tha the slices of beet are introduced into the diffu- sion batteries, a large percentage of the bacteria and yeasts occurring in the raw material is in active condition. In the case of raw sugars intro duced into the refinery the bacteria are largely in the form of spores which require many hours to germinate, even under ideal conditions. It also contains a small number of yeasts and mold spores, but just as in the case of the bacteria these form are also to a large extent in a dormant state of development. It is essential for their survival that they are in this condition, otherwise they would not be able to endure the low moisture level of the substrate and the effect of oxidation at the surface of the crystals. Hence, the danger of sugar losses through microbial growth in sugar re- fining is practically eliminated and the only vul- nerable stage, as far as microbial growth is con- cerned, is in the raw sugar washings, but both the temperature in which these liquors are maintained, as well as their constant circulation, reduce to a minimum any opportunities for microbial growth.

Flow Diagram of Boneblack Refining Process.

Courtesy of John Wiley & Sons, New York, Publishers of Spencer-Meade Cane Sugar Handbook, Eighth Edition

Briefly considered, the refining of cane su involves the following processes:

1. Affination or washing of the raw crystal
2. Dissolving or melting the washed sugar crystals.
3. Defecation
4. Filtration
5. Evaporation
6. Crystallization
7. Granulation
8. Bagging

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Chapter VII

THE MICROBIOLOGY OF SYRUPS

From a theoretical standpoint, both cane and beet syrup leaving the "multiple effects" have as a microbial flora only bacterial spores and possibly a small number of very resistant yeast cells which have survived the temperatures, incident to the evaporation of the clarified juices. In the raw sugar factory or the refinery, these surviving microorganisms are of no immediate concern, since the syrups are rarely kept for any length of time and pass continuously to the vacuum pan, where they are evaporated to a massecuite from which crystallized sugars are recovered. However, for the cane syrup manufacturer, the presence of viable yeast cells is of great significance, as the syrup has to be processed for distribution to the consumer, irrespective of the concentration of viable cells occurring in the product prior to the canning operations. From the microbiological standpoint the contamination of syrups is of no special significance, except where the product is to be canned and distributed as such for human consumption. It is, of course, obvious that this statement does not refer to the thermophilic bacteria, whose presence in the syrup is indicative of the numbers that may survive in the sugar that is crystallized from it.

The canning of cane syrups appears to be a very simple procedure and at the present time constitutes a very large industry, but there are a great many scientific points regarding it which are still not clearly understood in the Industry. It is a procedure which appears so simple and so uncomplicated that only the larger canners, who

have adequate laboratory control, are aware of the intricate points which have to be taken into consideration, if losses from swells are to be completely avoided. It is very confusing at times to those who are unfamiliar with the microbiological history of these products to discover that in almost all apparently sound cans of cane syrup there remains quite a large number of viable bacterial species. These species are almost invariably present in these products and yet are of no significance whatever, since they are incapable of developing at the density at which this material is concentrated. On the other hand, it is equally as confusing to find occasionally canned syrups which have undergone considerable decomposition, as measured by the degree of swelling of the cans, and yet contain no demonstrable evidences of the presence of viable yeasts. Since the processing temperatures that may be practically applied to the preservation of cane syrup are almost always below the temperature at which resistant bacteria are killed, it is not surprising that in any lot of sound cans of syrups one may obtain quite an appreciable growth of bacterial colonies on plates made from it. On the other hand, in canning molasses the temperatures are often below the therma death point of the species of yeasts contained in it and hence viable yeasts may be found in perfectly sound cans of this commodity. As a matter of fact, it is impractical to process molasses, especially the lower grades, at a temperature whic is adequate for the destruction of many of the species of yeasts normally occurring therein. It is proper, therefore, to apply the term of "inactivating temperatures" to the processing of cane molasses, as regards their yeast constituents, and the same might be applied to cane syrups as regards the viable bacteria which may be originally present in the raw material processed. The situation is further complicated by the fact that on occasions

when the canner is incurring considerable losses from the deterioration of his high grade cane syrups he is often convinced that this is the result of inefficient processing, whereas in many cases the processing temperatures have been too high and the decomposition is the result of excessive rather than of insufficient exposure to high temperatures. Acting upon this false assumption, as regards inadequate processing, the temperatures applied to all products are raised and the problem of swells is additionally aggravated. Still another complicating factor in the processing of cane syrups and blends of syrups with commercial glucose is that experience has shown that for every ten per cent addition of glucose (corn syrup) the temperature to which the blend has to be subjected for efficient sterilization is raised from five to ten degrees F. For example, where the processing temperature of a low grade molasses of 75 Brix density may be as low as 150° F. a blend of glucose and syrup at this same density would require a processing temperature of between 160 and 175° F. In the case of cane molasses processed at temperatures as low as 150° F., one has no difficulty in observing viable yeast cells even where the product has shown no deterioration in a six months' storage period and even where swells have taken place it will be found that the number of yeast cells is almost never sufficient to explain the decomposition. The evolution of gas and the swelling cans in these instances can be reasonably attributed to spontaneous decomposition to which all cane molasses are known to be susceptible. Another complicating factor in the procedures of canning of syrups and molasses is the frequent occurrences of swelling in syrups which have been processed at reasonably high temperatures and which have been stacked in the warehouses and which show evidences of fermentation within a few weeks time. This latter condition is usually assumed by the operators in

canning plants to be the result of fermentation and to indicate inadequate temperatures of processes. The microbiological investigations of these cases, however, almost always reveal the lack of viable yeasts and point unmistakably to a prolonged retention of heat in the cans. One of the evidences of the phenomena known as "stack burning" is the darkening of the syrup with a very pronounced reddening. This phenomena is almost entirely confined to the large or ten-pound cans and it is a condition which has been aggravated in recent years by the almost exclusive use of fiber cases instead of wooden ones previously used for this purpose. In an investigation many years ago of the causes of swells in syrups in a warehouse, the writer observed that even after two weeks of storage the syrup in large size cans in the closely packed warehouses had lost only approximately ten degrees in temperature. The cans were slightly puffed and a bacterial examination revealed that there were no yeasts present, but there were unmistakable signs of the syrup having been exposed for too long a period to high temperatures.

THE PROCEDURE FOR CANNING SYRUPS AND MOLASSES

The standard procedure for the canning of syrups and molasses differs from that of most food products in that the processing temperatures are applied to the product in bulk and distributed through fillers into the cans. In other words, the product is not processed in the cans, so the temperatures must be sufficient to sterilize the interior of the cans and inactivate or kill any viable yeasts which may be introduced into them before capping and sealing by the capping machines. In view of the fact that the cans are not cooled directly but retain their heat for some time, the

correct temperature of processing is that which is sufficiently high to inactivate yeasts during the period in which the can is cooling, after being sealed. It can be seen, therefore, that the larger size cans can be filled at much lower temperatures than the smaller ones and still have their contents preserved. Any excess of temperature of canning over that required to preserve the product from yeast growth is not only expensive but detrimental, as regards the color of the product processed. The canning temperatures for cane syrups and molasses and blends of cane syrups and glucose, which have been found to be effective, are given in the following table:

Product	Size of can Pounds	Temperature degrees F.
Pure Cane Syrup	10	160
"	5	165
"	2.5	175
"	1.5	180
Corn Syrup and Glucose (60-40) Blend	10	170
"	5	175
"	2.5	180
"	1.5	185
Second Molasses	10	155
"	5	160
"	2.5	165
"	1.5	165
Third Molasses	10	145
"	5	150
"	2.5	155
"	1.5	155

THE DEVELOPMENT OF VIABLE YEASTS IN CANNED MOLASSES

One of the most plausible explanations for the presence of viable yeast cells in sound and unswelled cans of canned molasses is that the inactivating effect of the temperatures of processing combined with the low oxygen tension prevailing in the cans prevents the organism from developing, although it may remain viable indefinitely. The writer has frequently detected a considerable number of viable yeasts in low grade molasses, which had been in storage for a period of a year or more, during which time it had given no evidences of any gas formation whatever. It is likely that the large amount of reducing sugars present in these products, together with the small amount of oxygen originally present after the cans have been sealed, prevents these yeasts from developing. If samples of canned molasses are taken and are transferred aseptically to an equal volume of sterile water in a sterile flask, a fermentation almost always occurs, thus indicating that the majority of the cans still contain viable yeasts. In fact, it is impractical to process low grade molasses for canning at a temperature which will insure the destruction of all living yeast cells, for when such temperatures are maintained the remedy proves worse than the disease for which it is intended to cure, because it gives rise to spontaneous evolution of gas and the swelling of cans (cf. chapter on The Deterioration of Molasses).

The efficient processing for canning of low grade molasses, however, demands a preliminary treatment which is sufficient to destroy all viable yeast cells and all vegetative forms of bacteria and all mold fungi. This preliminary processing is absolutely essential for the

prevention of swells due to the decomposition of these products and consists in diluting the molasses to approximately 70 Brix and evaporating it in open evaporators back to its original blending density, namely 75 Brix. By this treatment the unstable complex combination of reducing sugars and amino acids, which give rise to gas formations, are broken up and the product can then be processed at low temperatures and canned quite successfully. Although this heat treatment is sufficient to practically sterilize the product, it usually becomes contaminated in the lines between the evaporating plant and the canning rooms, so an additional processing is essential.

SWELLS DUE TO LEAKY CANS

It is not an infrequent occurrence that rather severe outbreaks of swelling in canned syrups occur where the history of the temperatures of processing and canning offers no clue to explain the causes for this condition. An examination of these cans reveals a large number of yeast cells and all of the physical characteristics of the products are unmistakably typical of fermentation as the cause of this condition. In these cases, the writer has observed that the contaminants were actually drawn into the can through leaky seams, which appears all the more unusual in view of the fact that often these swells have as much as five to ten pounds of internal pressure. An investigation of the causes of this condition revealed that the seams of the cans were defective and upon cooling, air would be drawn into the syrup and the seams would later be hermetically sealed by the internal pressure resulting from the fermentation. Where records show that the canning temperatures were adequately applied, in spite of which yeast growth occurs, the possibility of defective cans

being responsible is one which should never be disregarded.

The bacteriological control of syrup canning blends consists largely of supervision of processing and canning temperatures and since the cans are filled while hot an adequate supervision of the temperatures at which various products are introduced into the cans offers sufficient protection against losses from fermentation. In the writer's experience, this supervision is most essential, as regards the inspection for defective sealing of cans, for in many cases the capping machine may be operating over a short period of time ineffectively and the inadequately sealed cans may be set aside to be reprocessed and through an oversight the leaks may be repaired after the contents of the cans have fallen below the safety point for sealing. What is particularly needed in the Industry are recording thermometers that will be operative only when in contact with hot syrup, for the conventional recording instruments are not satisfactory, owing to the fact that frequently the Filler tanks are almost completely empty and the contact zone of the thermometer is exposed to air temperature. This gives a record of sudden changes in temperature which are unintelligible when applied to the temperature records of the canning operations. It would be fairly simple to devise an electrical recording thermometer which would record only when in contact with liquid and this would give, under normal operations, a discontinuous temperature line, but would be easy to interpret as a reference on any particular batches of syrups which have not spoiled in storage.

THE THERMAL DEATH POINT OF YEASTS OCCURRING IN CANE SYRUPS

The resistance of the various species of yeasts occurring in syrups to high temperatures is quite variable and the resistance of any single strain of species varies over wide limits, depending upon the density of the substrate in which it is grown, as well as the degree of dessication of the viable cells when they are introduced into syrup from dust in the atmosphere. The writer found a wide range of temperature resistance by more than fifty strains of yeasts, isolated from fermenting syrups. The resistance varied from a complete suppression of growth at 60° C. for ten minutes to a tolerance of 90° for the same period. Among the species of yeasts found occurring in syrups was Saccharomyces Zopfii, a species which had been previously discovered and completely studied by Artari (1). This species was first isolated from beet juice in a sugar factory in Saxony, where it was found to be associated with Leuconostoc mesenteroides. Artari determined the thermal death point of this yeast and found it to be about 67° C. and he regarded it as an unusually resistant species and gave as a comparison the following thermal death points of other yeasts:

Saccharomyces cerevisiae is killed by moist heat between 52-53
Saccharomyces ellipsoideus is killed by moist heat between 54-56
Saccharomyces Zopfii is killed by moist heat at 67° C.
Leuconostoc mesenteroides is killed at 87-88
Bacterium vernicosum (Zopf) is killed at 73-75

By Dry Heat

Leuconostoc mesenteroides is killed at 100-114

Bacterium vernicosum (Zopf) is killed at 115-120
Saccharomyces Zopfii is killed at 130-135

This is a very small yeast with a diameter of three to six microns and its size varies with the substrate in which it is grown. The measurements made by the writer on this species were as follows:

Series	Density of Substrate Brix	Diameter Microns	Length Microns
A	71.1	3.80	7.20
D	31.49	4.43	7.43

The writer, during a long period of research in the Syrup Canning Industry, isolated ten of the more prominent species of races of yeasts occurring in fermented canned syrups and molasses and made determinations of the variations in their thermal death point. A considerable amount of time was applied to the identification of these strains of yeasts, some of which were true Saccharomyces, others of which were Torulae (non-sporulating yeasts) and still others were Zygosaccharomyces. Some of the ten strains studied in that connection undoubtedly belonged to the same species and further study would probably have revealed sufficient differences to warrant placing them in separate taxanomic categories. The ten listed in the above table represented the most prevalent of all strains of yeast that occurred in the hundreds of cases examined by the writer. We may assume that many of the species identified in raw sugars and molasses also occur in cane syrups.

In addition to Saccharomyces Zopfii and the other strains of yeasts encountered in syrups, it

THERMAL DEATH POINT

S = True Saccharomyces
T = [illegible]
Z = Zygosaccharomyces

Yeast	70 Brix Cane Syrup Time - 10 minutes, Degrees F.							Diluted Syrup 50 Brix Time - 10 minutes, Degrees F.						
	[illegible]	60	70	[illegible]	[illegible]	[illegible]	100	00	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] (S)	+	+	+	+	+	+	-	+	+	+	+	-	-	-
Time required for development - days		(15)	(15)	(15)	(15)	(21)		(7)	(11)	[illegible]				
Culture I (S)	+	+	+	-	-	-	-	+	-	-	-	-	-	-
" II (S)	+	+	+	-	-	-	-	+	-	-	-	-	-	-
" III (T)	+	+	-	-	-	-	-	-	-	-	-	-	-	-
" IV (T)	+	-	-	-	-	-	-	-	-	-	-	-	-	-
" V (T)	+	+	-	-	-	-	-	+	-	-	-	-	-	-
" VI (T)	+	-	-	-	-	-	-	-	-	-	-	-	-	-
" VII (Z)	+	+	+	+	+	-	-	+	+	+	-	-	-	-
" VIII (Z)	+	+	-	-	-	-	-	+	-	-	-	-	-	-
" IX (Z)	+	-	-	-	-	-	-	-	-	-	-	-	-	-
" X (Z)	+	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = Growth
- = No growth

may be assumed that among these should be include Zygosaccharomyces nussbaumeri and Z. major, whic Hall, James and Nelson (2) found to constitute t predominant flora of Barbados molasses.

In determining the specific thermal death point of yeasts responsible for swells in cane syrups, attention must be given to the well known fact that the resistance on the part of yeasts t high temperatures is greatly influenced by the previous substrate in which the yeast has grown. Kayser (3) found that yeast cells withstood much higher temperature when in a dry rather than whe in a moist condition. In one case he found by experiment that a certain species of yeast, whic was killed at a temperature of 60 to 65° when in a moist condition, withstood a temperature, when dry, of 105 to 110°. The resistance of the spor was 10 to 20° higher than that of the vegetative cells. Henneberg (4) found that various races o yeast differed considerably in their resistance t heat. Saaz yeast ceased to bud at from 50 to 52° Frohberg yeast at 54 to 56° and Race XII at 58°. The cells stained, according to the staining age employed, as follows: Saaz at 58 to 60, Frohbert at 64, Race XII at 68 and Race II at 68 to 70. Schoenfeld and Rommel (5) showed that a certain yeast that had been grown for a long time in association with lactic acid bacteria possessed an unusually high resistance to heat. The resistance of this yeast was so high that it many time survived a temperature which killed the lactic acid bacteria with which it was infected. It was found that the yeast could withstand a temperatur of 65° for thirty minutes and the budding cells were more resistant than the old cells.

The writer has observed that in order to obtain consistent thermal death point results for the yeasts normally found in fermented canned

syrups it is necessary to culture them successively in sterile syrup over a range of at least five serial transfers. During this time the yeasts have acquired a constant resistance to temperature in their vegetative form, but even data so obtained does not always correspond to the actual exposure of the syrup in cans, where the gradation of drops in temperature on cooling is subject to frequent fluctuations.

As it may be generally assumed that the contamination of canned syrup is usually from the air at the Fillers, this would imply that the yeast cells causing the fermentation of hermetically sealed cans are air-borne and hence are in a more resistant condition, as a result of this dessication. Since the processing of syrups is usually above any conceivable thermal death point of yeasts, it is obvious that the contaminants that survive in the cans must either be acquired from the lines, from the cans, or from the air. In this connection, the writer has observed many instances where the contaminating yeast cells were derived from the caps of the cans. This contaminating material is frequently not brought into contact with the hot syrup until the temperature of the latter has dropped below the level at which all yeast cells are killed. In some cases the cans are maintained in a vertical position from the time they leave the Fillers until the time the cases are sent to the warehouses, in which event it is easy to see how contaminating material on the tops would be able to survive and later contaminate the can contents.

THE STORAGE OF SYRUPS FOR CANNING

In the larger commercial canning plants, the cane syrup is stored in very large tanks in cold-storage rooms, where the temperature maintained

varies from approximately 32 to 40° F. and usually at the former temperature. At this temperature, the fermentation of this product can be indefinitely inhibited, but here again appearances are often deceptive and as the writer has observed, syrups exposed to this temperature over a period of six to eight months may show no visible evidences of fermentation and yet contain a very large number of viable yeasts. Plate counts of many syrups which appeared to be entirely free from any fermentation may have counts ranging into the million and the cell concentration may often be at much higher levels than would be found in actively fermenting syrups at room temperatures. One of the contributing factors to this phenomenon is the greater retention of gas at the low temperatures, but even when these syrups are warmed to room temperatures the evolution of gas appears to be in many cases insignificant. It is evident, therefore, that the canning of cold-storage syrups may often present greater difficulties than in the canning of the fresh product. In the first place, the distributing lines may become highly contaminated with yeast cells and the chances of contamination in handling this material, both in the processing and canning rooms, are correspondingly increased. It is possible, also, that yeasts occurring in the syrups stored at low temperatures are actually higher than those occurring in the normal product.

Cane syrups may be preserved by other methods than by low temperatures and the writer developed a patented procedure, many years ago, for the preservation of these products by CO_2 pressure. It was found that at moderate levels of viable concentrations of yeast cells, this material could be preserved by the application of 45 pounds per square inch of CO_2 pressure at temperatures parallel to the optimum temperature for yeast growth.

This procedure, however, is only applicable to syrups in which no appreciable fermentation has taken place, for after fermentation has been initiated in these products the writer observed that pressures as high as 100 to 150 lbs. per square inch could result from yeast growth and hence it is obvious that at high concentrations of yeast cells no practical pressures of CO_2 could limit its further progress. One of the practical disadvantages of this process is that further processing is necessary after storage under gas pressure, in order to de-gas the syrup. This procedure might prove more economical than the maintenance of cold-storage plants and it has this further advantage that there is less oxidation and darkening of the product under CO_2 than under atmospheric pressures.

THE MICROBIOLOGY OF MAPLE SYRUP AND SUGARS

The microbiology of maple products differs radically from that of cane or beet juices, owing to the fact that the method of collecting the raw material is so different from that of the other sugar juices, which are industrially employed. As contrasted with the extraction of cane juice by milling, or beet juice by diffusion, where the rapid transmissal of the extracted juice to the clarifiers limits the period in which microbial growth can take place, no matter how heavily inoculated the extracted juice may have been, in the collection of maple juice the juice flows continuously through spouts inserted into the maple tree and because of the fact that the extrusion of the sap is very slow each particle is exposed over quite a long period to contamination and to temperatures at which microorganisms can develop. We are indebted to Edson (6), Fabian and Buskirk (7), Fabian and Hall (8) and to Fellers (9) for their

extensive investigations and published results on the microbiology of these products and to the recent publication and review by Hucker and Pederson (10) on this field of microbiology. Tanner's (11) work on the microbiology of foods also treats extensively of maple products and the author has made use of all of these publications in this section of our present chapter. The microorganisms associated with the deterioration of maple sap and maple syrups comprise bacteria, yeast and mold, for the density of maple sap is far lower than that of cane juices and the opportunities for the bacterial development in the former are, as we have already pointed out, much greater than in cane juice manufacture. As Tanner has pointed out, great improvements have been made in the production of maple syrup and the time intervening between the collection and the evaporation of the juice to the density of syrup is now much shorter, inasmuch as now each day's collection of sap is evaporated at the end of the day. The species of microorganisms which have been identified in maple sap are as follows:

I. BACTERIA
- Bacillus aceris (Edson, Jones and Carpenter (12))
- Bacillus parallelus (n. sp.)
- Pseudomonas fluorescens
- Aerobacter aerogenes (Fabian and Buskirk (13))

II. YEASTS
- Saccharomyces aceris-sacchari
- Saccharomyces behrensianus (Behrens) Klocker (14))
- Saccharomyces behrensianus (Hansen)
- Zygosaccharomyces mellis (Fabian and Quinet (15))
- Zygosaccharomyces barkeri (Barker)
- Zygosaccharomyces japonicus (Saito)

Zygosaccharomyces nussbaumeri
(Lochhead and Heron (16))

III. MOLD FUNGI

Torula saccharina (Heald and Pool (17))
Aspergillus (Fellers (18))

The deteriorative changes induced by microorganisms in maple syrup have been described and have been extensively studied and the following are of the most frequent occurrence. We are indebted to Tanner (19) for his review of these types of deterioration, resulting from the growth of microorganisms in maple syrup.

SOUR SAP

This condition refers to any abnormality in the product which imparts a decided odor and taste, without inducing any discoloration. Edson found saps which have undergone this deterioration to attain levels as high as 141,000,000 bacteria per cc. It may be assumed that this type of deterioration may be induced by a heterogenous contamination and may represent the competitive activities of a large number of species of microorganisms.

STRINGY SAP

This condition, which as the name implies refers to a stringy or a viscous product, has been found to result from the activities of an organism to which Edson gave the name Bacillus aceris.

(NOTE: It seems likely that some of the Leuconostoc species might also play some part in this type of deterioration).

GREEN SAP

This deterioration refers to deteriorative changes by microorganisms which impart a decided green or brownish green color to the product and Edson and Carpenter found the causative agent to be members of the Pseudomonas fluorescens group. This type of sap, according to Edson and Carpenter, is commonly called "buddy" sap and it was formerly believed to be correlated with the development of the tree in the Spring, that is Spring growth, but more recent investigations have shown it to be of bacterial origin. It seems possible that there may be a correlation between the change of seasonal variation in the composition of the sap and its susceptibility to the development of these specific bacteria.

RED SAP

This deterioration may be the result of pigmented microorganisms and has been found to be the result of the growth of certain chromogenic bacteria, although some pigmented yeasts are regarded as playing a constitutory part.

THE PROCESSING OF MAPLE SYRUP

Owing to the fact that the density to which the sap is evaporated in manufacturing maple syru is considerably below the level at which cane juices are evaporated, it might be supposed that there would be a correspondingly greater amount o deterioration, such as the swelling of cans that is so common in cane syrup canning operations.

The usual density of maple syrup is from 65-68 Brix and owing to its low density the product is susceptible to bacteria and yeast deterioration.

Strangely enough, maple syrup seems to be less susceptible to deterioration in cans than some of the other products of higher density. Among the microorganisms causing deterioration of maple syrup, we find Bacillus aceris, causing stringiness, Bacillus parallelus and also Pseudomonas fluorescens, causing "green sap". Aerobacter aerogenes has also been identified as the causative agent in some cases of ropiness in this product. It is not surprising, of course, that almost innumerable species of yeasts, particularly Torula, are capable of fermenting maple syrup and the species previously listed have been found to occur most prominently in these products. The canning temperature for maple syrups is approximately 90° C. and Fellers (20) observed that if the syrup is put in the cans at 93.3° C. (200° F.) and only a small headspace allowed in the can, the product may be kept indefinitely. An interesting observation on the simplicity of canning this product and its comparative immunity against spoilage in cans is that until recently most of the canned maple syrups were packed in screw-top cans, in which the chances of contamination are much greater than in the conventional method of sealing cane syrup cans. Possibly the lower colloidal content of maple syrup, as compared with cane, might be partly responsible for the greater ease of preventing microbial contamination.

One of the explanations for the greater ease at which maple syrup may be preserved by canning and the comparative freedom of the canned product from fermentation may be seen from the comparative analyses of cane and maple syrups. In the following table is shown the average analysis over a three-year period of syrups from thirteen Louisiana

factories and the analysis of maple syrup is taken from those given by Leach and representing an average over a long period of years for that product. It will be seen from this analysis that maple syrup in composition more nearly conforms to the pattern of white sugar syrups or liquid sugars, especially in its low ash content and its low level of colloid. The total sugar purities of the two products and the organic non-sugars, as shown in the table, will explain the differences in the greater ease of sterilization and maintenance of maple syrup, as compared to cane. It might appear to be rather paradoxical that maple syrup is less subject to microbial deterioration than cane, in view of the fact that its density is at a level at which both bacteria and yeast may develop, but this is probably offset by the higher processing temperatures that may apply to it, as contrasted to those levels of temperature to which cane syrup can be subjected with impunity, without forming decoloration products resulting from the decomposition of reducing sugars.

Product	Total Solids	Water	Ash	Sucrose	Reducing Sugars	Combined Sugars	Total Sugar Purity	Organic Non-Sugars	True Purity
Cane Syrup*	70	30	2.67	52.74	12.68	65.42	94.5	11.91	74.70
" "	71	29	2.80	55.11	8.62	63.73	89.7	4.47	77.62
" "	71.14	28.86	2.12	55.58	10.50	66.08	92.8	2.94	78.13
Maple Syrup**	64.30	35.70	0.53	63.36	0.42	63.78	100.0	0.0	98.5

*Owen, Wm. L. Cane Syrups and Molasses, Their Composition, Production and Consumption in the United States. Official Proceedings of the Assoc. of American Dairy Food and Drug Officials' 28th Annual Convention. August, 1924.

**Leach, A. E. Food Inspection and Analysis. John Wiley and Sons. First Edition. 1905.

THE PROCESSING OF CANE SYRUPS FOR IMPROVEMENT IN COLOR AND FLAVOR

Cane syrups vary widely both in color and in flavor, depending upon the areas in which the canes are grown, the type of soil, climatic conditions and to some extent upon the variety of cane from which the syrup is made. Owing largely to the fact that the volume of cane syrup produced annually is so variable and the industry is so frequently penalized economically by over-production, there have been practically no noteworthy developments on refining and processing the product for improvements of its flavor and in its zestfulness, as regards consumer demand. There are some areas in which only light-bodied syrups are popular and there are other areas in which cane syrup is practically unsalable, unless it is blended heavily with commercial glucose. It is not our purpose here to discuss consumer demand or enter into an enumeration of the various areas of the country in which special types of syrups find an outlet. It might be said, however, that one of the perplexing problems for the large refiner or blender of syrups is the fact that depending largely upon the prices of cotton, the output of syrups in some of the Gulf non-sugar producing states may fluctuate over wide levels. The writer has studied the correlation between cotton prices and syrup production over a long period of years and the results conclusively show that when the price of cotton is low, the production of cane syrups reaches enormous volumes, while in good cotton years the output is usually correspondingly low. This condition makes it very difficult for the refiner or blender of syrups to have any control of the markets and for this reason the incentive to produce very high quality, distinctive syrups has been lacking. The so-called refiners of cane syrup confine their operations as a rule to five procedures - (1) blending syrups of various types, (2) diluting them to

the lowest possible density acceptable under the standards of these products, (3) bleaching syrup to a brighter color for more appeal (4) marketing it under special attractive labels and (5) blending syrups with commercial glucose in various proportions.

The microbiology of cane syrups and molasses deserves mention under this particular heading, owing to the fact that investigations have shown that microorganisms, specifically yeasts, have a constructive role in the improvement of these products and their activities may not always be detrimental to the product. It has been the writer's experience over a long period of years of investigation of these products that a preliminary fermentation with certain specific yeasts, especially those isolated from molasses, tend to impart to syrups a much smoother flavor and some of the species impart a decidedly zestful and distinctive aroma, which would no doubt add to the consumer demand for syrups treated in this manner. That some of these products may owe their distinctive flavor to spontaneous fermentations which they may undergo is exemplified by the investigations of Hall, who found that the flavor and aroma of Barbados molasses is due to the presence of fermentation products formed by a species of bacteria which he isolated from molasses from that source and which he identified as Clostridium saccharolyticum. It was found that when this species of bacteria grows in molasses in the presence of yeasts the characteristic flavor is imparted, and it could be induced in molasses from other sources by inoculating them with it. One of the most general effects resulting from the growth of certain species of yeasts in syrups is an increased smoothness of the taste of the product even where no aromatic flavor is produced. This result is sometimes so striking that in many cases experienced syrup blenders may be deceived and suspect

the presence of considerable quantities of glucose in cane syrups that have undergone this fermentation. This smoothness in taste may be partially the result of decreasing the levulose in the product, but it is more than likely the result of some change in the amino nitrogen content of the syrups, resulting from the growth of yeasts and their utilization of this form of nitrogen.

THE MICROBIOLOGY OF WHITE SUGAR SYRUP (LIQUID SUGAR)

The industrial production of liquid sugars has attained very large proportions in recent years and the amount produced is increasing in volume annually and the areas which are being supplied with it are rapidly widening. This means that the product is being distributed over long distances and the prospects are that the industrial usages of the product will continue to increase and the shipping distances correspondingly extended. There are many conveniences and considerable savings in the use of liquid sugars, as compared with the granular product, because the cost of bagging and packaging is dispensed with and the cost of labor is correspondingly reduced, since the sugar in syrup form can be pumped directly from trucks and tank cars into receiving tanks. The saving in labor applies not only to the moving of liquid sugar, but also to the ease of handling it in the plant, to say nothing of the saving in time incident to the reduction of crystal sugar to a syrup form. One might also attribute certain advantages to the greater precision with which the industrial user of sugar can judge as to the amount of product one may add to or blend with the material which is being produced. One of the limitations, as regards the industrial handling and distribution of liquid sugar is that it is of course far more susceptible

to the growth of microorganisms than is crystal sugar. The life period of a sugar syrup, that is the length of time in which it can be stored at summer temperatures, and the distances that it may be transported without refrigeration depend upon many factors, the most important of which are:

1. The initial concentration of viable yeasts.
2. The chemical purity of the product particularly with reference to ash and invert sugars.
3. The particular species of yeast that it may initially contain.

Owing to the increasing production of this product, it becomes of paramount importance to the producers to apply bacteriological control in its manufacture and most important of all is the accurate appraisal of the level of yeast contamination on every shipment that leaves the plant. Liquid sugar, by virtue of its low density, is known to be quite vulnerable to microbial growth and one of the reasons why it has been so successfully distributed is that it is usually consumed rather promptly after it is produced. That is especially true during the summer months, as the product is not processed for the inactivation of any microorganism which it may contain and hence its keeping quality depends entirely upon its low microbial content, when manufactured, and its purity as regards elements of nutrition necessary for the development of yeasts. In order to attain the essential degree of purity, it is necessary to maintain the greatest possible cleanliness in the plant and to avoid any accumulation of contaminating material in pipe lines and tanks and in order to maintain the desired state of chemical purity, which is imperative, special methods of filtration of liquors have to be provided.

In well operated liquid sugar plants, where due precautions are taken to maintain cleanliness and sanitation and efficient preventive measures, the syrup as produced usually contains not more than approximately from 0 to 10 cells per cc., although at times the count may run as high as 100. The practical problem then is to be able to obtain an accurate and rapid appraisal of the level of viable yeasts in the product, before it is shipped to the customer. Unfortunately, this problem which seems so simple on the surface is by no means easy of attainment. In the first place, the time required to obtain the results from plate counts is the minimum of 72 hours, by which time in many cases the shipment has gone out of the plant before its degree of contamination has been ascertained. Direct microscopical counts are of course entirely untrustworthy when applied to the estimation at this level of yeast contamination. The writer has investigated over a period of years many procedures for obtaining trustworthy information on such syrups at the time of their manufacture. The procedures investigated were as follows:

1. Filtration of a large volume of syrup diluted with sterile water and filtered through Seitz filters.
2. Filtration through sterile filter aids.
3. Flotation method by treating the undiluted syrup with a suitable solvent and examining the supernatent liquid of the solvent.

Of all these methods, the flotation method, while not claimed to be entirely infallible, does offer a means of concentrating the yeast cells where they can be recognized by microscopic examination. This, of course, has its limitations in that it does not distinguish between the viable and non-viable yeast cells, but both the dead and living cells would be included in the appraisal

and regarded as a liability from the standpoint of the expected life cycle of storage of the product.

Where a manufacturer has a large number of industrial users of this product and where the material is used locally and shipped to some distances by rail, it is almost impossible to predict with any degree of certainty how well a lot of liquid syrup will hold up in storage, that is to say whether its freedom from contamination is sufficient for the storage period to which it will be subjected. For example, a shipment of the syrup, which analysis has shown contains as few as ten yeast cells per cc. and which is to be used locally, would seem to be sufficient guarantee that the material would not undergo any deterioration before it will be completely used up in a process. It may so happen, however, that products shipped to a local user might be subjected to more prolonged storage periods than one shipped hundreds of miles away, as plant operations in the first place might render it necessary to hold this material over for a considerable length of time. On the other hand, material shipped by tank cars may be used as soon as it is received and hence has no opportunity of deteriorating before it is processed. From a practical standpoint, therefore, it means that regardless of the prevailing temperatures or the season of the year, the product must be capable of resisting microbial development and fermentation resulting therefrom, for a period of several weeks, and to caution the industrial user against storing the product amounts to an adverse advertising program, especially in comparison with the crystal sugar which he had previously used. Not only is the bacteriological control difficult to apply in all cases, as regards using such data for infallible predictions as to the useful life period of the product, but investigations have shown that there are almost innumerable factors other than temperature which effect the growth rate of the

yeasts which it may contain. Some of the factors which have a tremendous bearing on the growth curve of yeasts in this product are (1) temperature, (2) influence of surface exposure, (3) influence of air, (4) effective agitation and (5) the nature of the surface of the containers in which it is stored. For example, the prevailing temperatures may be within optimum levels for the growth of yeast and the surface exposure may be so restricted that their rate may be actually lower than when stored at lower temperatures with more favorable surface exposure. As an example of some of these effects and influences, we give the following example of actual plate counts of syrups incubated at various temperatures:

Series	Temperature Degrees C.	Initial Count per cc.		1st Week
		Assumed	Actual	
A(1)	37	40	119	300
A(2)	37	40	119	300
B(1)	34	40	119	300
B(2)	34	40	119	300
C(1)	30 (approx.)	40	119	400
C(2)	30 "	40	119	400
D(1)	10	40	119	23
D(2)	10	40	119	3

In order to determine the rate of growth of yeasts in syrups containing varying initial counts, the following experiments were carried out. In these experiments, metal cylinders were used with the inside walls coated with a special coating which is used in large tanks in the commercial

storage of liquid sugars. The capacity of these cylinders was approximately 1500 cc's and they were provided with tightly fitting rubber stoppers. The syrups were inoculated so as to attain various levels of viable cell concentration per cc. and bacteriological analyses were made at weekly intervals. It will be noted that where the initial counts were less than 20 per cc. there was practically no increase after a three-weeks period. Where the counts were 80 per cc., the growth was rapid from the beginning and in two weeks time there was sufficient evidence of deterioration, which would have indicated incipient deterioration by the appearance and taste of the product. (See table on page 107.)

The growth curves vary considerably, depending upon the source of the syrup and its chemical composition, which is illustrated in the following table where a comparison is made of four different syrups from different sources. These experiments were conducted also in metal cylinders and the densities of the product were approximately the same. (See table on page 108.)

It will be seen from the following table that in Sample B the yeast growth started promptly and proceeded to increase for seven weeks. At the end of this time it was heavily clouded and showed every evidence of deterioration. Sample A showed no yeast growth until about the fifth week, while Sample B showed considerable growth at the end of the third week.

The growth of yeasts in refined syrups is largely confined to the surface and not only is growth first observed at the surface and around the surface contact of the product with the container, but it seems likely from our investigations that the yeast cells tend to colonize in localized

Count per cc.					Count after Incubation					
Series	Inoculum Added	Estimated	Actual	Concentration Average	1 wk.	Average	2 wks.	Average	3 wks.	Average
A (1)	0.0	0.03	1.0	0.5	1.0	0.5	1.0	0.5	1.0	1.0
(2)	0.0	0.03	0.0		0.0		0.0		1.0	
B (1)	0.0	0.3	1.0	0.5	0.0	0.0	1.0	0.5	1.0	1.0
(2)	0.0	0.3	0.0		0.0		1.0		1.0	
C (1)	0.25	5.0	17.0	20.0	7.0	36.0	4.0	68.0	4.0	3500.0
(2)	0.25	5.0	24.0		65.0		132.0		7100.0	
D (1)	0.5	10.0	34.0	60.0	32.0	206.0	100.0	5100.0	200.0	15,225.0
	0.0	10.0	88.0		380.0		10,100.0		30,250.0	

Sample	Yeast Count								Appearance
	1st Wk.	2nd Wk.	3rd Wk.	5th Wk.		6th Wk.		7th Wk.	
				Before Reinoculation	After Reinoculation	Before Mixing	After Mixing	Before Mixing	
A	0	0	0	0	500	9,200		6,700	Slightly Cloudy
				Before Mixing	After Mixing				
B	4	201	13,650	5,800	3,700	3,100		3,700	"
				Before Reinoculation	After Reinoculation				
C	0	0	3	2	700	5,600		4,500	"
				Before Mixing	After Mixing				
D	340	900	9,150	7,450	11,800	13,250		29,500	Heavily Cloudy

areas on the surface of the liquid. It also appears that regardless of where the contaminating material is introduced into the syrup, it tends to migrate eventually to the surface and frequently columns of syrups which have been purposely inoculated in the lower layers would eventually be found to contain yeast cells only at the surface of the column. In order to determine this migration of yeasts to the surface in contaminated syrups, the writer designed special glass cylinders to be used in the investigation of this characteristic of the growth of yeast in products. These cylinders were approximately of the following dimensions:

Height ..... 18 inches

Diameter ..... 1 7/8 inches

The cylinders were provided with four glass outlets sealed into the outer walls of the vessels and equipped with stopcocks with the outlets spaced at equal distances on the sides of the cylinders at approximately five inch intervals. These cylinders were sterilized and were filled to within two inches of the top with sterile syrup. The contents of the cylinders were then inoculated through the outlets with a syrup containing a known number of viable yeast cells. The cylinders were then incubated at 34° C. over a ten-day period. The following table shows the results and that in spite of the fact that the syrups were inoculated at the bottom of the cylinder, the yeasts were concentrated at the top in one week's time:

(See table on following page.)

SYRUP INOCULATED AT BOTTOM OF CYLINDER

Level at which Sample Taken	No. of Yeasts per cc	
	1 Week	10 Days
Top	43,000	76,000
1st Outlet (1.5" from Surface)	1,000	3,500
2nd Outlet (6.0" " ")	0	200
3rd Outlet (11.0" " ")	0	375
4th Outlet (16.0" " ")	0	1,800

In the next experiment the syrup was inoculated at the middle of the column and the results were very much the same.

SYRUP INOCULATED IN MIDDLE OF COLUMN

17 Day Incubation Period	
Surface	206,000
1st Outlet	----
2nd Outlet	200
3rd Outlet	----
Bottom	100

These results tend to prove what is commonly experienced in practice and that is that the surface is the greatest zone of activity for contaminants and whenever this can be protected the rate of deterioration in the product can be greatly reduced. This characteristic of the yeasts occurrin in liquid sugar introduces a problem in the accurate appraisal of the level of viable yeasts in any

lot of syrup that is to be shipped, inasmuch as we obtain much higher results from the surface than from points below the surface and to agitate a syrup of this density, sufficient to get uniform distribution, is very difficult. On the other hand, it seems likely that it is perhaps just as well to obtain the density of cells at the surface rather than an average throughout the liquid, after the material has been thoroughly mixed. This characteristic of the formation and migration of yeasts in these products emphasizes the comparative ease with which surfaces may be protected and deterioration correspondingly diminished.

INFLUENCES OF THE INERT GASES UPON THE GROWTH OF YEASTS IN LIQUID SUGARS

It may be readily conceived that since the yeasts causing the fermentation and the deterioration of liquid sugars are so aerobic in their oxygen demands that if the air at the surface of these syrups could be displaced by inert gases, such as CO_2 or nitrogen, the storage period of these products could be greatly prolonged, even at fairly elevated temperatures.

Among the most critical factors affecting the susceptibility of liquid sugars in storage to deterioration are the condensation of moisture at the surface, resulting from changes in temperature, and the contamination of the exposed surface of yeast from air. Schmalz (21) has pointed out the importance of these factors and the utilization of ultra violet surface irradiation to counteract them. In addition to this method of protection, advantages can be derived from the use of air filters, or breathers, on the storage tanks and the maintenance of reduced surface areas, by the use of storage vessels with a low ratio of surface

to total volume, that is the tall narrow type, as opposed to the large diameter tanks having a high ratio of surface to volume.

METHODS OF LABORATORY CONTROL OF CANE SYRUPS AND LIQUID SUGARS AND MOLASSES

For the estimation of the number of viable yeast cells in cane syrups and molasses, the following substrates have been found to be most suitable and further modifications have not shown any definite advantages or improvements either in the total counts obtained or in the time required for yeast development. In order to screen out the bacteria which ordinarily occur in these products and whose presence is of no significance, it is important to use a substrate of sufficient density to retard their development. The media found best suited to this purpose are the following:

CANE SYRUP AGAR

Cane Syrup diluted to 50 Brix with Tap Water	1 Liter
Peptone	5 grams
Agar Agar	15 grams

MOLASSES AGAR

Blackstrap Molasses diluted to 50 Brix with water	1 Liter
Peptone	5 grams
Agar Agar	20 grams

de Whalley and Scarr (22) recommend a special medium of the following formula, for the enumeration of yeast in sugars or syrups and have found it particularly valuable in that it retards the growth of mould fungi.

20.00	grams	liquid malt extract (A. & H.) containing 25 percent water
0.78	"	peptone B. D. H.
2.67	"	dextrin B. D. H.
2.37	"	glycerin B. D. H., sp. gr. 1.26
1.00	"	ammonium chloride B. D. H.
15.00	"	powdered dried agar B. D. H.
1200.00	"	(1 liter) 45 Brix sucrose syrup containing 8 to 10 percent invert sugar on sample

These media are particularly useful for the enumeration of the species occurring in sugars, syrups and molasses, which Henrici (23) terms "osmophilic", owing to their ability to grow in highly concentrated solutions of sugars. The conventional malt agar recommended for these estimations of yeasts in dairy products and for foods is not well adapted for use in the above connection, as bacterial growth is not suppressed and accurate yeast counts where both bacteria and yeasts occur are difficult to obtain.

CANE JUICE AND SYRUP AGAR

This medium is identical with the one given above, except that 10 percent of cane juice is added to the syrup, prior to its dilution with water to 50 brix. The cane juice used should be unclarified except by filtration through cheese cloth or a fine strainer. (Cf Chapter V.)

IRRADIATION OF LIQUID SUGARS

It is well known that the depth of penetration of ultra-violet rays is quite limited even in clear liquids and that their germicidal power is restricted to a very shallow depth. In order to determine the effective range of light of this wave length upon liquid sugars, the following tests were made.

Ultra-violet light of approximately 2600 A.U. was applied to the surface of white sugar-syrup samples, distributed to various depths in Petri dishes. The depths of the samples varied from one-eighth of an inch to one inch and the time of exposure was for five minutes and the distance from the light source was six inches. The original count of yeast in the sample was 200 per cc. The results are given below.

Series	Depth of Syrup	Treatment	Count in one week	Count in three weeks
			per cc.	per cc.
A (1)	1/8"	Radiated	24	1
A (2)	1/8"	Non-Radiated	200	200
B (1)	1/2"	Radiated	100	100
B (2)	1/2"	Non-radiated	300	11,800
C (1)	1"	Radiated	700	4,000
C (2)	1"	Non-radiated	500	6,300

From the above results, it will be noted that irradiation is effective up to one inch depth of syrup, but at the latter depth it does not materially reduce the yeast count. However, since it so happens that most of the yeast development in this product is at the surface, irradiation should

prove most effective in the control of its deterioration during storage.

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Chapter VIII

THE MICROBIOLOGY OF RAW SUGARS

The microorganisms occurring in raw sugars at the time of their manufacture may be classified as to origin as follows:

1. Those originating in the juice and which survive the various stages of processing incident to their manufacture, hence it is obvious that only the sporogenous species of bacteria are included in this category.

2. Microbial contaminants from the air which gain access to the sugar in the centrifugals or are derived from contaminating material in the granulators or during the bagging process.

3. Microorganisms derived from the bags in which the product is packed.

4. Sporogenous species of bacteria which develop at the temperatures at which some of the intermediate products, particularly syrups, are held over during processing. This group consists of the thermophilic bacteria which are of particular importance, as effecting the quality of refined sugars used for canning purposes.

The microflora of raw sugars almost invariably includes the three principle groups of microorganisms, namely:

1. Fungi
2. Bacteria
3. Yeasts (including both the pseudo and true forms)

All of the species that have so far been isolated from sugars have been included in our discussion of the microflora of cane and beet juices and cane syrups.

The microbial population of raw sugars varies greatly, depending upon the conditions of manufacture and also upon the moisture levels of the finished product. This subject has been extensively studied by Deerr and Norris (1), Ammons (2), Tempany and De Charmoy (3), Church (4), the Kopeloffs (5), the writer (6) and more recently by De Whalley and Scarr (7). As to the number of microorganisms in raw sugars, the Kopeloffs made an extensive study of the microbial levels of populations in bagged raw sugars and have contributed very valuable data on the correlation between sugar deterioration and the total number of organisms present. (See following tables.)

SUMMARY SHOWING CORRELATION BETWEEN DETERIORATION AND NUMBER OF MICROORGANISMS

Mark	No.	Part of Bag	Sampling	Loss S. P.	Gain R. S.	No. of Microorganisms
Am.	1	Middle	3rd	2.5	2.30	120,000
		Surface	3rd	3.5	1.93	4,900
Cun.	4	Surface	3rd	3.0	1.63	20,000
		Middle	3rd	3.2	1.58	100,000
Am.	5	Middle	3rd	2.0	1.60	115,000
Am.	3	Middle	3rd	2.3	1.38	50,000
Am.	3	Surface	3rd	1.8	1.38	4,000
Am.	3	Middle	3rd	1.0	1.31	65,000
F.	1	Middle	3rd	1.8	1.30	1,650,000
Am.	5	Middle	2nd	1.6	1.23	70,000
Am.	6	Middle	3rd	1.2	1.22	120,000
Am.	1	Middle	2nd	1.2	1.10	4,000
M.	3	Middle	3rd	1.1	1.05	100,000
Am.	3	Middle	2nd	0.5	1.02	30,000

SUMMARY SHOWING MAXIMUM NUMBERS OF MICROORGANISMS WHERE NO DETERIORATION OCCURS

Mark	No.	Part of Bag	No. of Microorganisms
P.	1	Middle	6,000
		Surface	7,000
P.	2	Middle	6,000
		Surface	8,000
P.	3	Middle	2,100
		Surface	22,000
C.	1	Middle	8,000
		Surface	1,100
C.	3	Middle	5,000
		Surface	14,000
C.	6	Middle	22,000
		Surface	1,000
Agr.	1	Middle	3,100
Agr.	3	Middle	1,800
		Surface	300
Cab.	2	Middle	2,000
		Surface	500
Cab.	3	Surface	110,000
M.	3	Middle	1,050
		Surface	370
Ag.	1	Surface	2,600
Ag.	2	Surface	4,500
Pil.	1	Surface	24,500

De Whalley and Scarr, more recently, have subjected the matter of microflora of normal and abnormal raw sugars to an extensive investigation, using special substrates, and their results are shown in the following tables: (See tables following)

NORMAL RAW SUGARS

Country of Origin	No.	Nutrient Agar pH 6.8			Bacto Wort Agar pH 4.8			Osmophilic yeast pH 4.7		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
Cuba	1	350	0	5	50	0	1	0	150	0
	3	30	0	0	0	0	0	0	0	0
	4	170	0	0	80	10	0	0	100	0
San Domingo	5	170	0	0	40	5	0	0	45	0
	7	80	0	0	0	0	0	0	10	0
	8	680	60	1000	0	0	7600	0	30	80
Haiti	10	325	0	0	0	0	0	0	10	0
B. W. Indies	12	2400	200	0	875	0	0	0	700	0
U. K. Beet	13	250	0	0	0	0	0	0	0	0

ABNORMAL RAW SUGARS

Country of Origin	No.	Nutrient Agar pH 6.8			Bacto Wort Agar pH 4.8			Osmophilic Yeast Agar pH 4.7		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
Cuba	2	120	2800	0	20	410	0	0	12,400	0
San Domingo	6	4,750	45	5	2980	20	50	0	7,000	20
	8	680	60	1000	0	0	7600	0	30	80
Haiti	11	12,000	150	70	10	0	290	0	300	80

It will be seen from these tables that the abnormal sugars have a very high level of concentration of osmophilic yeasts, as compared with the normal sugars, and the above authors draw the conclusion that these species of yeasts play a very vital part in the deterioration of syrups, as well as raw sugars. In their investigation of the role played by these yeasts, they give the following microbial counts of raw beet syrups of approximately 72 brix.

% water	Mesophils	Osmophils
28.7	2,300	4,000
28.2	150	1,000
29.0	16,000	12,800
28.0	15,000	8,000
28.6	900	4,000
29.6	3,500	7,600
29.1	5,160	17,600
29.1	2,560	12,000
29.4	1,000	850
Average 28.8	5,070	7,600

De Whalley and Scarr classify the micro-organisms occurring in raw sugars under the following categories:

BACTERIA

B. subtilis
B. mesentericus vulgatus
B. aerogenes
B. coli, pyocyaneus
Sacrina citrica
Staph. albus
Saccharomyces sp.
Penicillium 5 sp.
Rhodotorula
Actinomyces sp.

YEASTS

Zygosaccharomyces
Schizosaccharomyces
Torulopsis

FUNGI

Mucor racemosus

These authors have pointed out in their publication on this subject that the ability of yeast to survive in high density sugar liquors and to induce a fermentation of it may be an acquired characteristic and in their tests on the acclimatization of strains of Saccharomyces cerevisiae they were able to induce this zymogenic faculty in a strain of brewers yeast in a period of three months of serial transfer to increasing sugar concentration to an extent that the strain of yeast employed could induce within a three-hour incubation period an active fermentation of a 45 Brix sugar solution. These authors give the following comparison between the fermentation ability of the acclimated yeast, (2) the original strain and (3) the same unacclimated strain that had been cultivated under conditions to form ascopspores, as follows:

Substrate	Time required to Induce Fermentation
1. Acclimated Strain	3 hours
2. Original Strain	1 week
3. Same after forming ascospores.	Very feeble after one week.

From this evidence of the acquired ability of yeast of specialized adaptation to light substrates, such as brewery mashes, to acquire the ability to grow in high density sugar solutions, it may be inferred that many of the species of true yeasts that are found in sugars may have originated from sources entirely alien, thereto.

In consideration of the peculiar physical conditions of sugars, it is interesting to observe that many investigators have consistently found non-sporulating species of bacteria in both raw

and granulated sugars and the presence of these forms may seem to be difficult to explain in a substrate so low in moisture and so nearly anhydrous in its physical composition. The presence of E. coli in semi-refined sugars, such as plantation, granulated and Turbinado sugars, has sometimes been attributed to unsanitary conditions in the plants in which they were produced and health authorities have not infrequently assumed that the origin of such species were from the wash waters used in the centrifugals. As a matter of fact, E. coli or a species strongly resembling it is a constituent of the epiphytic flora of most of the grasses and cereals and is very probably, also, in the flora of sugar cane. It is not surprising, in view of its distribution in nature, that Scarr and de Whalley detected it in raw sugars. While in some respects it might seem surprising that such species of bacteria survive in the dry environment offered by crystals of raw sugars, in others this material is more conducive to their survival than dry dust would be. The molasses moisture film surrounding the crystals of these sugars is never completely exhausted and dehydrated, though it may fluctuate with the atmospheric moisture levels in the environment in which the sugar is stored. Where the density of the film increases and its osmotic levels become higher, the protoplasma of the bacterial cells become gradually adjusted by degrees and thereby retain their viability. This chance of survival is enhanced by virtue of the fact that these contaminants are most probably introduced into the sugar dust at the centrifugal or in dust in which a large percentage of sugar is occluded and hence the change of osmotic pressure of their old and new environment is not too severe.'

While the presence in either raw or refined sugars of these adventitious bacteria of low thermal tolerance is of no possible significance

as regards any changes which they might induce, they are of significance in serving as a criterion of the cleanliness of the surroundings in which the sugar containing them is manufactured. Since they originate principally as air-borne contamination, the extent of their numbers in any sugars reflects the cleanliness of the premises where the sugar is manufactured and hence refined, granulated sugars are theoretically no more immune to their presence than is the raw product.

THE CHANGES IN COMPOSITION OF RAW SUGARS DURING STORAGE

Raw sugars are known to be susceptible to a variety of changes in composition during storage, the pattern of these changes being predicated upon the nature of the microorganisms inducing them, or to the chemical composition of the product where spontaneous decomposition is responsible for the changes in the product during storage. The characteristic changes in composition of raw sugars as induced by microorganisms may be classified as follows:

1. Typical inversion of sucrose in the molasses films with a corresponding increase in reducing sugars. This form of deterioration is characteristic of the deterioration induced by mold fungi.

2. Decreases in sucrose values, accompanied by decreases in reducing sugars. This form of deterioration is characteristic of a mixed flora of mold fungi and yeast, where inversion of sucrose precedes the fermentation of invert sugars.

3. An increase in polarization, as a result of the fermentation of levulose by yeast.

In addition to the above changes there are, as is now well known, purely spontaneous decompositions that take place in raw sugars and which are the result of reactions that will be discussed at length in a later portion of this publication. (cf Chapter on Deterioration of Blackstrap Molasses)

DETERIORATION OF RAW SUGARS IN STORAGE

The history of this subject is so extensive and its bibliography so voluminous that it is difficult to cite all of the literature that has been published on it and we can only review the most significant of the contributions that have been made towards the solution of the problem. For it cannot be doubted that the problem has been entirely solved and that the knowledge of the causes of sugar deterioration, as well as means for its prevention, is now almost universally known and practiced. At the present time, the manufacturer of raw sugars can appraise the storage quality of his sugars and can predict with considerable accuracy what their behavior will be, when stored under even partially favorable conditions. Notwithstanding the fact that the problem is no longer of any particular economic significance, a review of the evolution of our knowledge of the subject is of more than academic interest, since it reveals what is of great importance and that is the limitations and zymogenic capacities of the microbial flora of raw sugars. And it might be said, in justification of an extensive review of this subject in the present connection, that raw sugars are becoming progressively more difficult to refine and that it is the common experience of refiners that very old sugars, even though they reveal no evidences by chemical analysis of having been impaired in refining value, do, nevertheless, respond inadequately to refining

procedures and hence yield greatly reduced returns in the refined product. It is interesting to review the successive steps by which this knowledge of the predictable behavior of raw sugars in storage was acquired and the investigations through which this knowledge was derived. It was the bacteria in sugars that first commanded the attention of the bacteriologists and mycologists and R. Greig Smith (8) of New South Wales, Australia, was probably the first to study the bacterial species of raw sugars and to attribute to them a role in sugar deterioration. Contemporaneously, if not coincidentally, was the work of Dodson (9), who published actual data showing the deterioration of sugars resulting from their inoculation with suspension of bacterial spores from cultures of bacteria isolated from raw sugars. About the same time, Deerr and Norris (10), of the Hawaiian Sugar Planters Experiment Station, published the results of their investigation of the causes of sugar deterioration and assigned to certain bacterial species, which they had isolated, the faculty of inducing these changes. This investigation was followed by that of Deerr and Brain (11), who made an extensive investigation of the bacterial flora of Hawaiian sugars and described the cultural and morphological characters of the bacteria constituting it. Owen (12) further pursued this study of the bacterial flora of sugars and established the conditions under which bacterial action upon sugars could occur. As a result of the latter studies, it was for the first time conclusively established that the bacterial species occurring in sugars could not be responsible for the changes in normal raw sugars during storage, as had been attributed to them by previous investigators. All of the investigators who had taken part in these researches up to this point had contributed very useful data on the typical products formed from the action upon sucrose of the species of bacteria occurring in sugars and the knowledge that was

derived regarding the origin of gum levan and the conditions conducing, thereto, was of great value in connection with an understanding of the chemistry of this reaction. It might seem strange that bacteria, rather than yeast or mold fungi, had so preoccupied the attention of investigators up to this point in their search for the causative agents in sugar deterioration. This was all the more surprising, since Shorey (13) had at a much earlier date attributed this phenomenon to the activities of a species of mold fungi, namely Penecillium glaucum. However, the explanation is not difficult to find, since the bacterial species indigenous to sugars, both raw and refined, are prodigious and prolific in their growth and are certainly the most conspicuous of any of the constituents of the microflora of sugars. While a few colonies of mold fungi developing upon plates of substrate inoculated with dilutions of a raw sugar might be interpreted as of exotic origin, the numerous and typical gummy or viscous colonies of bacteria that always developed were highly indicative of their importance in relation to the changes in the composition of the sugar that was under investigation.

In retrospect, however, it seems surprising that once the bacteriological investigation of the flora of raw sugars had become popular, that the bacterial constituent of their microflora should have so entirely pre-empted the field of investigation. As early as the last decade of the previous century, Kammerling of Java (14) had isolated many species of mold fungi from comparatively dry sugar and had assigned to them a distinctive role in inducing sugar deterioration and Scott (15) detected the presence of both Penecillia and Spergilli in sugar liquors and determined their inverting powers on sucrose in high concentrations and prescribed precautionary measures for their control.

Somewhat later, Ammons (16), who was probably the first to determine the ability of pure cultures of mold fungi to induce inversion of crystal sugars, inoculated raw sugars with a suspension of mold spores, but his observations were confined to the difference in rate of moisture absorption by the inoculated and control samples of the sugars used in his experiments. Browne (17), who was the pioneer in the chemical investigations of the changes in the composition of sugars in storage, studied a large number of species of Torulae and Monilia occurring in raw sugars and ascribed, to a new species which he isolated therefrom, unique powers of inverting sucrose in sugars of normal composition, these two species, viz. Monilia nigra and fusca. However, his study did not include actual inoculation tests upon sterile, raw sugars, with these species of suspected deteriorative agents.

The extensive investigations of the Kopeloffs (18), which were applied both to the study of the species of mold fungi occurring in sugars and their deteriorative powers and an improvement in measures to be applied in their enumeration conclusively established the significant relationship between the concentration of mold in sugars and the rate of the deterioration of the latter. Preventive measures were outlined for the manufacture and the storage of raw sugars and the zones of dispersion of microbial contaminants in different portions of the bags were subjected to a thorough appraisal.

The avenues of contamination of sugars had already been thoroughly appraised by Church (19) in her study of the microbial flora of the products and the environment of sugar manufacture and the value of the Kopeloffs' investigations was largely that of appraising the potential deteriorative proclivities of all of the species of mold fungi

occurring in raw sugars. The Kopeloffs (20) endeavored to correlate the species and number of mold fungi with the degree of deterioration that would be induced in sugars, just as Tempany and De Charmoy (21) had previously done with the species of Torulae which they found to occur in plantation white sugars. There are several factors that militate against a satisfactory correlation of this kind, not the least of which is the difficulty of adequately sterilizing a sugar without greatly changing its physical characteristics. The moisture that may be lost may be restored later, but the molasses film is invariably altered in some respects and added moisture is not always as suitable for the growth of the microorganisms in sugars as is that which is absorbed naturally on the periphery of the molasses film surrounding the sugar crystals. It is for this reason that results of actual inoculation of sterilized sugars have been far from consistent and although the uses of various gaseous methods of sterilization have been proposed, these, as a rule, are ineffective for the most part and where adequate for the purpose of sterilization almost invariably alter the composition of the sugars to which they are applied.

Another factor that greatly confuses the interpretation of the correlation between the concentration of microorganisms in sugars and their deterioration in storage is the question of what the microbial count as ascertained really signifies. As regards the concentration of mold fungi per gram, it is well known that the zymogenic potential of these microorganisms is correlated with the extent and area of the mycelia, rather than in the concentration of spores, and hence a colony assay means very little in appraising the extent of inverting potential that may be present in any portion assayed. It is possible to have a

low count of spores of fungi and still have a dangerous concentration of mycelia present in sugars. For example, the Kopeloffs (22) found a maximum of thirty-three percent of the total microorganisms in raw sugars to consist of mold fungi, when the total per gram of all microorganisms was only 230, but in all cases where the total count was in the hundreds of thousands the percentage of mold was entirely insignificant.

If the mold fungi count in raw sugars is always far below what would be regarded as the minimum required to produce significant fermentative changes in the composition of the sugars in which they occur, the yeast count, while much higher, also seems incommensurate with the changes that take place. On the other hand, for those investigators who have attributed sugar deterioration to the activities of true and false yeasts, which are always present in raw sugars, the dilemma is equally apparent as regards the number of viable yeasts occurring therein. In view of the fact that even the largest concentration of viable yeasts that have been reported as occurring in sugar seem entirely inadequate to explain the degree of chemical changes that these products undergo in storage, this is one of the least assailable arguments against the theory that yeasts are responsible for this phenomenon. For example, Rahn (23) has calculated mathematically that it requires a concentration of from 20-90 million cells of S. lactis to induce perceptive coagulation of milk and several million yeasts to induce perceptible concentrations of alcohol from sugars. Owen (24) obtained maximum values for yeast concentrations in inoculated molasses of approximately 9,000,000 cells per gram and in raw sugars filmed with this material a maximum of 33,000 yeast cells per gram. It should be borne in mind, in this connection, that the concentration of microorganisms

in sugars should actually be calculated on the molasses constituting the film around the sugar crystals, which seldom exceeds five percent of the total weight. Hence, a count of 33,000 per gram of raw sugar actually represents a concentration of approximately 700,000 cells per gram of fermentable substrate. Even in abnormal sugars, de Whalley and Scarr (25) obtained maximum counts of yeasts of approximately 78,000 per gram of sugar. Here again, we must take into consideration that the microorganisms in sugars are not uniformly distributed throughout the mass of crystals but tend to be concentrated and stratified around false grain or in particles of dust adhering to some of the crystals principally at the surface of the sugars in the centrifugal. Hence, a count on a small sample from a bag of sugar merely represents the average of some highly contaminated aggregates of crystals and others that have a very low degree of microbial contaminants. Since the mold fungi appraisals do not give a true index of the extent or the area of mycelia of fungi present and the yeast contaminants are known to be very unequally distributed throughout any mass of sugars, the information acquired from quantitative bacteriological determinations leave much to be desired in determining the deteriorative potency of any lot of raw sugars. The Kopeloffs (26) endeavored to show that the degree of contamination of a sugar frequently determined its behavior in storage and that sugars which would ordinarily resist deteriorative changes, owing to their low moisture content and high density films, would, nevertheless, succumb, thereto, if its initial contamination exceeded a certain level of concentration. We will consider this aspect of the subject more in detail in a later portion of this publication, where the "Factor of Safety" of sugars is discussed.

TYPICAL DETERIORATIVE CHANGES INDUCED BY MOLD FUNGI IN RAW SUGARS DURING STORAGE

The typical pattern of deterioration of raw sugars in storage, as induced by mold fungi and free from the complicated pattern that sometimes results from the associated action of yeasts, reveals merely a decrease in polarization with increases in reducing sugars and usually some increase in moisture. The latter increase usually reflects the increasing hygroscopic nature of sugars, as a result of the increase in its reducing sugar content. In the following table, by Browne (27), is presented the characteristic behavior of sugars stored over a long period, with the typical deteriorative changes induced by the normal microbial flora of these products. (See table on following page.)

In the above data, the increase in reducing sugars and the decrease in polarization and clerget values of the samples will be noted and also the small changes in their moisture content over a period of two years or more.

The characteristic behavior of sugars artificially and heavily inoculated with mold fungi can be seen in the following table from the author's (28) published results on this subject. (See table on page 134.)

It will be seen in the above table that the deterioration in a raw sugar induced by pure cultures of mold fungi, over a one-month storage period, is almost equivalent to the corresponding values shown in the previous table on sugars stored over a period of several years.

(BROWNE, C. A. THE DETERIORATION OF RAW CANE SUGAR, A PROBLEM IN FOOD CONSERVATION. JI OF IND. AND ENGR. CHEM., VOL. 10, NO. 3, 178, 1918)

Date of Analysis	Sample	Polarization	Water (a) Percent	Sucrose (a) by Clerget Percent	Invert Sugar Percent	Ash Percent	Total Nitrogen Percent	Reducing Power Percent
May, 1915	A	96.0	1.10	95.69	0.81	0.41	0.00	0.00
	B	96.0	1.00	95.39	1.00	0.00	0.00	0.00
	C	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	D	96.00	0.00	96.00	0.00	0.00	0.00	0.00
Average		96.00	0.00	0.00	0.00	0.00	0.00	0.00
October, 1915	A	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	B	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	C	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	D	96.00	0.00	96.00	0.00	0.00	0.00	0.00
Average		96.00	0.00	96.00	0.00	0.00	0.00	0.00
January, 1916	A	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	B	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	C	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	D	96.00	0.00	96.00	0.00	0.00	0.00	0.00
Average		96.00	0.00	96.00	0.00	0.00	0.00	0.00
August, 1917	A	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	B	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	C	96.00	0.00	96.00	0.00	0.00	0.00	0.00
	D	96.00	0.00	96.00	0.00	0.00	0.00	0.00
Average		96.00	0.00	96.00	0.00	0.00	0.00	0.00

BEGINNING OF EXPERIMENT

No.	Moisture Percent	Single Polarization	Sucrose Clerget	Sucrose Clerget moisture free basis	Reducing Sugars	Safety Factor
7	1.20	96.6	97.75	98.93	0.93	0.533
10	1.20	97.1	97.72	98.90	1.25	0.526
13	1.25	96.1	96.76	97.98	0.95	0.385
14	1.35	95.4	96.00	97.31	1.40	0.337
15	1.35	96.2	96.42	97.73	....	0.377
16	1.30	97.1	97.12	98.39	....	0.451
20	1.20	96.1	96.68	97.85	1.02	0.361
25	1.00	96.3	97.04	98.02	0.88	0.337
26	1.25	96.2	96.66	97.80	0.97	0.374
27	1.20	95.9	96.23	97.39	1.00	0.318
40	1.50	94.7	95.14	96.57	1.30	0.308
43	1.50	94.7	95.12	96.56	1.70	0.307
44	1.75	94.9	95.28	96.97	1.80	0.370
46	1.35	94.1	95.08	96.38	20.5	0.274

ANALYSIS AFTER ONE MONTH

No.	Moisture Percent	Single Polarization	Sucrose Clerget	Sucrose Clerget moisture free basis	Reducing Sugars	Deterioration % Sucrose Clerget m.f.b.
7	1.40	95.3	95.65	97.00	2.00	1.9
10	1.50	94.4	95.00	96.44	3.16	2.5
13	1.45	95.4	94.92	96.31	1.60	1.6
14	1.35	94.9	95.19	96.49	1.40	0.9
15	1.50	94.4	94.55	95.90	1.96	1.8
16	1.35	94.7	94.87	96.16	3.03	2.1
20	1.15	95.5	95.68	96.79	1.04	1.1
25	1.00	96.0	96.26	97.23	1.37	0.8
26	1.00	94.7	95.38	96.34	1.51	1.5
27	0.90	95.3	95.59	96.45	1.66	0.9
40	1.25	93.5	94.46	95.65	1.90	0.9
43	1.80	93.6	93.23	94.93	2.40	1.6
44	1.15	94.0	94.03	95.12	2.60	1.8
46	1.80	93.0	93.05	94.75	3.17	1.6

DETERIORATIVE CHANGES IN RAW SUGARS AS INDUCED BY YEASTS OR TORULAE

We have observed that the typical pattern of the deterioration of raw sugars as induced by mold fungi is a simple inversion of sucrose with the formation of theoretical amounts of invert sugars and usually with an increase in the moisture content of the sugars, as a result of the greater predisposition to moisture absorption by the more hygroscopic properties of the levulose formed. We not infrequently encounter, however, a quite dissimilar pattern of change in the composition of raw sugars where the reducing sugars decrease and the polarization increases and where the moisture content tends to drop at a rate parallel to the loss of invert sugar. This pattern of change in raw sugars in storage has long been observed and it was noted by Tempany and Watt (29), by Noel Deerr and Norris (30) and commented upon extensively by Browne (31). Owen (32) subjected the phenomenon to an extensive investigation and developed procedures for the artificial inoculation of raw sugars with pure cultures of torula strains, which had exhibited unusual zymogenic powers on invert sugars in raw sugars of normal moisture content. In the investigations referred to, it was found that as long as the destruction by yeasts of invert sugar in a molasses film of raw sugar is in progress, the carbon dioxide formed offers great protection against the inversion of sucrose by mold fungi and as a rule it is only when the source of this gas is exhausted that further deterioration takes place.

The pattern of the type of fermentation of raw sugars may be illustrated in the following table from one of the writer's previous publications and it shows the effect of filming a sugar with a molasses filming coating of molasses heavily inoculated with a Torula culture. (See table on following page.)

Sample	Moisture %	S. P.	S. C.	S. P. dry basis	S. C. dry basis	Detn.	R. S.	Moisture Increased or Decreased
Cuban No. 1 (1)	0.5	98.1	98.24	98.73	98.66	....	0.86	-0.2
" (2)	0.25	98.2	98.15	98.39	98.66	....	0.86	-0.5
Control (1)	0.87	96.2	96.43	97.27	97.60	....	1.49	-0.98
" (2)	1.00	96.3	96.42	97.39	97.60	....	1.44	-0.85
Inoc. Film (1)	0.55	97.8	98.02	98.56	97.94	....	1.04	-1.12
" (2)	0.60	97.8	97.86	98.45	97.94	....	1.00	-1.07
			Two Weeks' Run					
Cuban No. 1	0.92	96.5	96.63	97.50	98.66	0.8	1.00	+0.6
Control Film	2.52	94.8	94.82	97.16	97.90	0.74	1.19	+0.8
Inoc. Film	1.57	96.8	96.70	98.24	98.04	....	0.90	-0.6
			Three Weeks' Run					
Control Film	4.55	94.1	94.42	98.90	97.80			
Inoc. Film	2.60	96.8	97.12	99.70	98.70			+1.50
			Four Weeks' Run					
Inoc. Film	0.30	99.8	99.01	99.40				

The period of this experiment was three weeks and in that time it will be noted that the reducing sugar of the sample had been completely destroyed in the inoculated film samples. The decreased affinity for moisture, resulting from the elimination of reducing sugars, is shown in the loss of moisture. From the results of his extensive investigation of the protective action of Torulae against the deteriorative action of fungi, Owen (33) proposed a procedure for the filming of raws with cultures of the more active of the species of Torulae normally occurring therein. An example of the gain in value of a raw sugar filmed in this manner is shown in the following table, the values having been calculated on the sliding scale basis for the purchase of raws in the U. S. at the time the investigations were conducted.

Sample	Initial increase over control deg.pol.	Value of inc. per ton raw sugar	Final increase over control deg.pol.	Value per ton	Final increase over original deg.pol.	Value per ton	Net increase over control per ton
I (1)	0.60	$1.08	1.50	$2.79	0	$....	$2.10
(2)	0.50	0.90	2.00	3.60	0.30	0.54	2.80
(3)	1.10	1.98	2.70	4.86	0.60	1.08	3.34
(4)	0.60	1.08	3.30	5.94	1.60	1.44	5.50
II (1)	0.95	1.71	1.40	1.12	1.50	2.70	0.82
(2)	0.80	1.42	1.85 0.70	3.30 0.56	1.20	2.16	2.62
(3)	0.90	1.62	2.40 1.60	4.32 3.20	2.30 2.00	4.14 3.60	3.04
III (1)	2.40	4.32					
(2)	1.90	3.42	4.00 3.50	7.20 6.30	0.50 1.00	0.40 0.80	5.60 5.18
(3)	0.70	1.26	2.60 3.20	4.68 5.76		1.45	

While there would be some loss of value from the loss of weight incident to the fermentation of invert sugar by the Torulae, the gain derived from the increase in polarization would more than compensate for it. There is an additional advantage, also, from the refiners' standpoint, resulting from the better refining quality of the sugars so treated. Under the present methods of raw sugar manufacture, where lime clarification is used, the manufacture of good-keeping raws is achieved by the crystallization of sucrose from the lowest possible massecuite purities. The result is a raw sugar in which the crystals are coated with very low purity molasses and a product which is not only difficult to refine, but one which upon storage becomes increasingly difficult to refine, owing to purely autogenous changes which occur in the films surrounding the crystals. (cf Chapter on Molasses Deterioration).

Opinions as to the ability of the Saccharomycetic constituents of the microflora of raw sugars to induce the deterioration of the latter have been rather divided (34) and while the author has encountered no instance where deterioration of raw sugars could be validly attributed to yeast action alone, Browne (35) showed that the inverting power upon sucrose of Monilia nigra, which he isolated from Cuban Raws, was very pronounced. However, the Monilia are more nearly related to the fungi than to the yeast. In the latest systems of classification, this group is included under the fungi, although the medical mycologists still refer to them as yeast and regard them as essentially a yeast-like organism occurring on skin and lesions and which form mycelium in nutrient solutions of low nutrient content. In their recent publication, de Whalley and Scarr (36) attribute to the yeast in raw sugars and syrups a very significant role in sugar deterioration, although data from experiments

under controlled conditions is lacking and only the loss of total sugars in raw syrups is offered to substantiate this inference. They base their conclusions largely upon the amount of alcohol formed from the fermentation of syrups and the high concentrations of yeast that were found therein. These authors report an unusual instance of the activity of yeast in a cargo of Mauritius molasses sugar in the hold of a ship, where the evolution of CO_2 was so pronounced that the stevedores unloading it were all overcome by it.

It is difficult to obtain valid proof of the deteriorative propensities of the species of Saccharomycetes occurring in sugars and it is quite easy to attribute to them a definite and important role in almost any instance where deterioration has occurred. In the first place, they are apt to be more numerous or to be revealed in larger numbers in the bacteriological analysis of the sample than are the mold fungi, which are difficult to appraise, as regards the area occupied by the mycelia and the colonies produced on plates. On the other hand, it is quite easy to obtain very definite evidences of the inverting power of many of these species of yeast in high purity syrups diluted below 70 Brix density, and it is from such tests that most of the inferences as to their deteriorative power on sugars have been obtained. The difficulty of sterilizing sugars without seriously altering their molasses films has militated against the acquisition of valid proof of the role that is to be assigned to yeast in the deterioration of raw sugars, whereas the deteriorative powers of mold fungi are now generally known by all investigators in this field.

An example of the behavior of one of the Torulae species occurring in sugars which were investigated by the writer is shown in the following table, from which may be noted the rapid

destruction of reducing sugars in a molasses diluted to 68 Brix and the increase in purity, resulting therefrom.

Sample	Corrected Brix	S. P.	S. C.	R. S.	True Purity	Percent CO_2	Torulae per gram
Control, 1st Week	68.19	40.8	44.06	12.3	64.6	.....	
Inoc., 1st Week	66.94	42.8	46.05	11.1	67.6	.....	2,560,000
" 2nd Week	67.07	46.4	48.85	10.0	72.8	0.0365	3,800,000
" 3rd Week	68.26	45.2	46.06	9.6	67.4	0.0560	9,440,000
" 4th Week	65.68	48.0	46.83	9.2	71.3	.....	7,520,000

THE APPRAISAL OF THE SUSCEPTIBILITY OF RAW SUGARS TO DETERIORATION

The evolution of the ways and means for the appraisal of the predictable keeping quality of raw sugars undoubtedly contributed more to the solution of the economic problem of losses from deterioration than all of the knowledge that had been acquired from the extensive investigations of the microorganisms responsible for it. The difficulty of artificially sterilizing raw sugars made it almost impossible to appraise the deteriorative abilities of the species of yeast, fungi and bacteria which were known to constitute the flora of these products and the practical impossibility of determining, by either chemical or physical analyses, the nature of the composition of the film surrounding raw sugar crystals, made predictions as to the probable behavior of sugars in storage entirely impossible. Owen (37) attempted to apply to the problem the conventional methods of absorbing in glycerine the films of raw sugars, in an effort to appraise their densities, but this procedure was found to be inapplicable to the solution of the problem. In the meantime, the Colonial Sugar Company of Australia had evolved a formula

(38) that seemed to have proven adequate for the purposes and was based upon the fact that the ratio between the non-sugars and the moisture in any unwashed raw sugars established the density of the film and hence its relative susceptibility to the growth of microorganisms and the deterioration that they might induce. It is evident that the purity of the massecuite from which sugar is crystallized determines the density of the film surrounding the crystals, for the stability of final molasses has always been recognized and the deteriorative propensities of high grade syrups to deterioration had been known from the earliest times. Owen (39) subjected this appraisal to an extensive investigation and offered as an explanation of the validity of the "Factor of Safety", the theory that it connotes a film of molasses exceeding the density at which the microorganisms in sugar could develop.

Browne (40), who was probably the first to apply this Factor to an extensive and scientific investigation, concluded that for the average Cuban and Puerto Rican sugar the original Factor of $\frac{W}{100-S} = 0.333$ should read $\frac{W}{100-S} = 0.30$ and the values should be based upon the true clerget sucrose, rather than upon polarization. The original Factor was based upon polarization, rather than upon sucrose clerget values for sucrose, so in lowering the Factor to 0.30 and applying it on the true, rather than apparent, sucrose purity the predictable behavior of a raw sugar can be very adequately appraised.

In his published work on this subject Owen (41) discussed the comparative susceptibilities of raw sugars produced by the two and the three massecuite systems and the comparative validities of Factors of Safety of the sugars produced thereby. He called attention to the fact that in discussing the composition of the films surrounding

raw sugar crystals we are inevitably led to a consideration of the system of sugar boiling practice, for upon this more than anything else does the composition of the films depend. In the manufacture of raw cane sugars one of two systems of sugar boiling is usually practiced; viz., the three-massecuite system or the two-massecuite system.

The three-massecuite system is as follows: A first massecuite is made of nearly pure syrup, say of 80-85 purity, molasses 60-65; a mixed massecuite of 72-75 purity, molasses 48-50; a low grade crystallizer massecuite, 60 purity, molasses exhausted.

The two-massecuite system consists of the following procedure: A first massecuite is made of 75-78 purity, molasses 54-55; a second massecuite is made of 60 purity, with molasses exhausted.

On the whole, the three-massecuite system is the more popular in the tropics and is regarded as the better of the two, but when studied with reference to the susceptibility of molasses films to deterioration it is open to an objection which we will endeavor to point out. Under the three-massecuite system, we have a sugar made from the first massecuite, which has crystallized from a high purity syrup, and the molasses films are therefore of a correspondingly high purity and unsafe "factor". It is quite true that in this system first molasses is sometimes added to reduce the purity of the syrup, but usually this is only done when the purity is above 87. It is quite obvious that the crystals produced from this massecuite are none too well protected by their films of high purity molasses. The crystals produced from the mixed massecuites are much safer and it is quite common to mix the two sugars after they

are dried. Now it is just here that an apparent exception to the operation of the safety factor is introduced, for a mechanical mixture of safe and unsafe crystals may appear safe from an average analysis, but the unsafe crystals are not rendered safer by the proximity of the safe crystals in the mixture. It is important to remember in this connection that the protection of the non-sucrose moisture ratio must be an inherent quality of each individual film, rather than of the whole mass. There are many unmistakable instances of the deterioration of sugars conforming to the safety factor, which is to be attributed to the fact that the sugar under observation was a mechanical mixture of safe and unsafe crystals.

If we consider the relative merits of the two systems of sugar boiling with the sugars produced in the two cases, our first impression may be still further supported. The pertinent inquiry in this connection is, which will be the more hygroscopic: a sugar coated with a small amount of high purity molasses as those from the first massecuite in the three-massecuite system, or those with larger amounts of low purity molasses, as in the case of the two-massecuite system? We might naturally conclude that the lower purity molasses would be the more hygroscopic, but if we pursue our inquiry still further and inquire as to which sugar would first be likely to attain an unsafe non-sucrose moisture ratio we would be inclined to predict that the high purity films would sooner become unsafe, because the amount of moisture required to render them so is so much less than in the other case. In the following table are shown the percentages of molasses of varying purities necessary to bring the polarization of sucrose crystals to 96 and the "factors" of the sugars resulting therefrom. (See Table on following page)

Polarization	85 Brix 38.25 Sucrose 45 Purity			83.75 Brix 41.88 Sucrose Molasses 50 Purity			82.5 Brix 43.375 Sucrose 55			81.25 Brix 48.75 Sucrose 60			80 Brix 52% Sucrose 65		
	Percent Mol.	Moisture	Factor	Percent Mol.	Moisture	Factor	Percent Mol.	Moisture	Factor	Percent Mol.	Moisture	Factor	Percent Mol.	Moisture	Factor
94.00	9.71	1.46	0.243	10.32	1.68	0.280	10.98	1.92	0.320	11.71	2.20	0.366	12.49	2.50	0.417
94.5	8.91	1.34	0.243	9.46	1.54	0.280	10.07	1.76	0.320	10.73	2.00	0.364	11.45	2.29	0.417
95.00	8.10	1.215	0.243	8.60	1.40	0.280	9.15	1.60	0.320	9.76	1.82	0.364	10.41	2.08	0.416
95.50	7.29	1.094	0.243	7.74	1.26	0.280	8.23	1.44	0.320	8.78	1.64	0.364	9.37	1.87	0.416
96.00	6.48	0.372	0.243	6.88	1.12	0.280	7.32	1.28	0.320	7.80	1.46	0.365	8.33	1.66	0.415
96.50	5.67	0.85	0.243	6.02	0.98	0.280	6.41	1.12	0.320	6.83	1.28	0.366	7.29	1.46	0.417
97.00	4.86	0.729	0.243	5.16	0.84	0.280	5.492	0.96	0.320	5.85	1.10	0.366	6.25	1.25	0.417
97.5													5.20	1.04	0.416
98.0													4.16	0.83	0.415

This table calculated - assuming sugar grains 100% sucrose.

The resulting sugars being pure grains coated with molasses of varying purities: then a sugar

$$\text{Pol. } 94 - \frac{100}{9.71\% \text{ Mol.}} \times 38.25 = 3.714$$

$$= \frac{90.29}{94.004} \text{ P. of sug.}$$

The Brix and sucrose to correspond to molasses of varying purities assumed. No water used in centrifugals.

Figures compiled by F. P. Brennerman (42)

Taking the polarization of 96 degrees for the purpose of comparison, we find that it requires 8.33 per cent of a molasses of 65 purity to give that polarization and the sugar will have a "factor" of 0.415, while with a 49 purity molasses 5.67 per cent will give the same polarization, but will yield a sugar with a "factor" of 0.283. It is of interest to note from this table that the "factor" resulting from the addition of the same molasses is constant, irrespective of the amounts added. In other words, the "factor of safety" refers in the last analysis to the molasses film and not to the crystals themselves.

There are several factors that tend to invalidate the "factor of safety" as an index of the keeping qualities of raw sugars, some of which tend to give a distorted picture of the real susceptibility of the molasses film in revealing its high susceptibility to deterioration when it actually is less susceptible than if it corresponded to the accepted values of $\frac{W}{100-P} = 0.50$, while others would show an acceptable "factor", when in reality it is more predisposed than otherwise to deterioration. The first of these two conditions may be induced by a destruction of invert sugar in the film by yeast, thus decreasing the values of the 100-P and increasing the ratio of moisture to non-sucrose solids. As a result, a sugar, by virtue of losing non-sucrose solids, has its "factor" increased, but by virtue of the impregnation of the films of molasses with CO_2 it may be far less susceptible than the original sugar was before this fermentation occurred. On the other hand, we often encounter a sugar having a safe "factor" by virtue of having already undergone some deterioration, by mold fungi, and having sustained some loss of sucrose and a gain in invert sugar. As a result, the ratio of moisture to non-sucrose solids may be lowered and a "safe factor"

acquired, thereby. Obviously, since the density of the molasses film in such cases is only slightly increased by virtue of the molecular transformation of sucrose into dextrose and levulose, there is, of course, a slight increase in the osmotic values of the resultant hexose sugars formed, but in the early stages of deterioration this would be a negligible consideration. Of course, theoretically this situation can only occur where the moisture content of the sugars is at equilibrium with the moisture content of the outside air, for otherwise the greater hygroscopicity of the invert sugar formed would cause more moisture to be absorbed and an increase in the moisture non-sucrose ratio. Many sugars which have undergone some incipient deterioration may be adjudged safe for storage, when actually they may be very susceptible to such changes. The Kopeloffs (43) suggested that the "factor of safety" is valid within certain definite limits of contamination with mold fungi and that when these levels are exceeded the moisture non-sucrose ratio is no longer a valid index of keeping quality. Here again, it is probable that in such exceptions the high mold count may be parallel with deteriorative changes that have already taken place and by virtue of which a satisfactory "factor of safety" has been acquired. It is obvious, of course, that the "safety factor" can be applied only to unwashed raw sugars, since its validity is integrated with the composition of an undiluted molasses film of normal density. Once this normal pattern is changed by washing the crystals with water, the interpretations of its significance become entirely untrustworthy.

There is still another factor that may tend to invalidate the "factor of safety" as a criterion of the behavior of raw sugars in storage, although so far as the writer is aware it has never been accurately appraised. This factor is the state or condition in which the absorbed moisture film

surrounding the sugar crystals is held. There may be a peripheral edge of the film which may be considerably lower in density than is the interior portion of the film. It is conceivable that on this peripheral zone the conditions might be favorable for mold growth, although the ratio of moisture to non-sucrose solids might reveal by analysis no material change in the "safety factor" of the sugar under examination.

The problem of raw sugar deterioration in storage is no longer an economic problem and instances of any material losses occurring from it are extremely rare, as the contributory causes are now too well recognized to ignore the precautions known to be necessary for its prevention. Browne (44) estimated that the losses from the 1916 Cuban crop, as a result of deterioration, amounted to one and a half million dollars. However, since that time vast improvements have been made in warehousing and in handling of raws, but the greatest factor has been in the manufacture of sugar that more closely conforms to the approved concept of the "factor of safety". While raw sugars have been progressively made more stable, as regards their keeping quality, they have unfortunately acquired this status by virtue of becoming increasingly more difficult to refine. Their stability has been imparted by the incorporation of the cyrstals within a molasses film of increasingly lowered purity and higher density and viscosity. And in addition, the refiner has the additional woes incident to the chemical changes of an autogenous and spontaneous nature which take place in raw sugars that have remained for long periods in storage. A knowledge of the microbiological activities that occur in raw sugars has contributed greatly to the successful and efficient processing of other products of a similar nature. Molasses feeds, for example, present the same and identical

problem in that it is not the total moisture content of the finished product, but the final density of the molasses in which the feed is incorporated that determines its keeping quality. For example, de Whalley and Scarr (45) cite examples of products of this kind deteriorating from yeast fermentation to a point where all of the sugars in the molasses had been exhausted. However, the moisture content of the feed in these cases was 23 percent at the time of its manufacture, while safe practice prescribes a maximum moisture content of approximately 15 percent.

SUMMARY

We may summarize the foregoing discussion of the factors effecting the deterioration of sugars as follows:

1. The deterioration of raw sugars is almost exclusively confined to changes in the composition of the films surrounding the sugar crystals.

2. For sugars to conform to the "factor of safety" they must be crystallized from syrups of such purity as to yield molasses films of a density which is beyond the limits in which mold growth is possible.

3. The "factor of safety" to which raw sugars must conform, in order to be insusceptible to deterioration, varies with the species and numbers of microorganisms present.

4. The "factor of safety" is inapplicable to washed sugars and to those that have already undergone deterioration and also to mechanical mixtures of sugars of unsafe and safe "factors".

5. Under the three-massecuite system the sugar made from the high purity syrup is likely to prove more susceptible to deterioration than the sugars made from the mixed massecuites.

THE MICROBIOLOGY OF REFINED SUGARS

From our present knowledge of the microflora of white sugars, including both plantation granulated and refined, we may assume that the microorganisms constituting it are identical with that of raws, varying from it only in degrees, as the numbers of viable microorganisms found in the former are smaller, as would be expected, than in the latter. Of the various species of microorganisms that are brought into the refinery with the raws, some of them eventually find their way to the finished product and survive there. The spores of the bacteria entering the refinery with the sugar survive the clarification and the evaporation processes, although their numbers are greatly reduced by settling and filtration of clarified liquors. The yeasts that are introduced into the process with the raw sugars find their way to the finished product by the same avenues as they entered the raws, namely by dust-borne contamination. The mold fungi are also derived from the air and dust in the surroundings and occasionally their concentration in the refined product exceeds the levels of the raws from which they were made. Considering the fact that the mold constituent of the microflora of either raw or refined sugar is exclusively air-borne, this condition is neither unique nor surprising. There is, however, quite a difference in the significance of high levels of microbial contamination in the two products, for in raw sugars the presence of high concentrations of microorganisms is of no particular importance, so long as the moisture non-sucrose

ratio is kept within the bounds of safety. In refined sugars, on the other hand, the presence of a large number of any of the groups of microorganisms is always significant, since these may impart to the products in which the sugar is used undesirable properties, or induce fermentations that may be quite costly. It is in the category of "carriers of microorganisms", rather than victims of their action, that the microbiology of refined or white sugars in general is of economic interest and concern. For just as the storage qualities of raw sugars depend entirely upon their conformation to the "factor of safety", so the resistance of direct consumption sugars depends for their imperishability upon the almost complete dryness of the product. The limits of moisture that a refined sugar can carry are almost negligible, for even were the "factor of safety" applicable to these products, which it is not, the permissable moisture content of a refined granulated polarizing, for example 99.5, would be only 0.15 percent. There is no safety limit, as regards susceptibility to deterioration of a solution of sucrose and water, and even a super-saturated solution is subject to the action of mold fungi and yeasts. The storage quality of any refined sugar depends (1) upon its low moisture content, (2) its resistance towards moisture absorption and (3) upon the conditions in which it is stored.

The perishability of direct consumption sugars, when improperly stored, is now so generally recognized that risks are seldom taken in the Industry in the storage of this product or in its handling after it leaves the granulators in the refineries in which it is stored. It is now almost common practice to equip granulated sugar warehouses with air conditioning devices that keep the humidity and the temperatures on levels consistent with that outside the building. The important factor is to keep the air in the warehouses

always slightly warmer than outside, so that there will not be a tendency for the moisture in the air to condense on the surface of the sugar. The temperature control is just as important, also, as a means for the prevention of the sugar caking and setting "in the bags and forming hard lumps". A free running sugar is highly important for every industry, as the labor required for breaking up the contents of bags of sugars which set like concrete is a costly operation.

Since refined sugars may carry sufficient numbers of fungi and yeasts, and sometimes even mesophilic bacteria, to induce undesirable fermentations in foods or syrups or therapeutic products in which they are used, the bacteriological control in sugar refining is obviously important. There are frequent instances where even mesophilic microorganisms carried by highly refined sugars may present a very real problem in its industrial utilization in the manufacture of certain food products.

THE MICROFLORA OF REFINED SUGARS

As we have previously pointed out, white sugars, either plantation granulated or bone-black refined raws, present almost an identical pattern, as regards the microflora with which they are contaminated. The groups of microorganisms occurring in refined sugars are as follows:

1. Bacteria, Mesophilic and Thermophilic
2. Mold Fungi
3. Yeasts, Pseudo (Torulae) and True Saccharomycetes.

Owen (46), as a result of extensive investigations of the bacteriology of sugars used by

clients in the beverage, dairy and baking industries, recommended definite standards for the microbial purity of refined sugars. The standards that he proposed were as follows:

1. That the percentage of mold infections of any lot of refined sugars should not exceed 20.

2. That the number of mold spores per gram should never exceed 10.

3. That not more than 20 percent should contain viable yeast cells.

4. That the number of yeasts present per gram should not be more than 50.

5. That the average number of bacteria, other than thermophilic (standards for which are already in use) should not be over 50 and the maximum tolerated should be 100.

These standards are vital in the procurement of an acceptable quality of sugars for the beverage industries, where the sugar is the principal ingredient and where the finished product is not sterilized and where the sole protection against fermentation is the carbonation that is applied to the product. As a result of an investigation of a severe epidemic of deterioration of refined granulated sugars, which had been stored for several years in an inadequately ventilated warehouse, the writer concluded that refined sugars may be a real menace as a carrier of mold fungi, bacteria and yeast in the beverage industry particularly.

In these samples of highly contaminated sugars, the mesophilic counts were as follows:

Number	Number of Organisms		
	Bacteria	Molds	Yeasts
1	?	10	6,000
2	20	15	0
3	25	15	10
4	100	40	40
5	40	0	10
6	20	15	15
7	30	0	10
8	150	20	60
9	10	20	0
10	5	25	10
11	5	690	10
12	10	30	10
13	10	20	0
14	15	0	0
15	5	0	10
16	20	5	15
17	10	0	10
18	20	0	5
19	10	150	5
20	70	0	10
21	70	30	0
22	10	5	0
23	10	5	5
24	15	0	0
25	20	25	5
26	30	5	50
27	20	20	5
28	70	5	10
29	20	0	0
30	30	0	15
31	20	0	10
32	25	5	0
33	30	0	5
34	0	0	10
35	30	0	15
36	5	0	10
37	10	15	5
38	5	0	0
39	20	0	0
40	5	10	0
41	5	0	0

From the table on page 153, it will be observed that the numbers of yeasts and molds occurring in some of these sugars are much larger than the average for raw sugars. In fact, the analysis of some of these samples makes it questionable whether they would be suitable for use in beverages and food products. It is interesting, also, to observe in this table that there is no correlation whatever between the number of bacteria and of molds and yeasts.

THERMOPHILIC BACTERIA IN REFINED SUGARS

While the acceptance of the theory of the mesophilic microorganisms carried by refined or direct consumption sugars being a contributory factor in the spoilage of beverages or food still awaits action on the part of industrial users of this commodity, the indictment of it as a proven carrier of destructive thermophilic bacteria has been thoroughly established. It was two decades ago that the National Canners Association published the results of their investigation on the causes of spoilage in canned corn and established the relationship between the incidence of this spoilage and the presence of thermophilic bacteria in the refined sugar used for the purpose. In 1928, Cameron and Williams (47) published their results on the occurrence of species of these bacteria in refined sugars and subsequently Cameron and his co-workers (48) of the National Canners Laboratories and James (49) of the Bureau of Chemistry of the U. S. Department of Agriculture traced the incidences of spoilage in canned foods, particularly corn, to the presence of viable spores of thermophilic bacteria in the sugars used. Rarely has there ever been an indictment of a food product that appeared as groundless as this one appeared to its manufacturers, for the refined sugar gave every appearance o

chemical and bacteriological purity, as judged from its physical and chemical composition. How could there be ascribed to it qualities that were so alien to its appearance and to its past reputation? If the indictment was spectacular and amazing and surprising, the proof was nonetheless irrefutable, for by an extended series of canning tests the incidence of spoilage from the use of refined sugars that had been proven by bacteriological analyses to be comparatively free from thermophilic spores was so low, as compared with that occurring where high count sugars were used, that any doubt as to the role played by contaminated sugars in this spoilage was unqualifiedly dispelled. The following table is taken from their published results, as shown below:

SPOILAGE IN NO. 2 CANS OF CORN DUE TO THE PRESENCE OF THERMOPHILIC ANAEROBIC BACTERIA IN SUGAR

Processed at 250 Deg.F.	Percent "swell" spoilage in corn with		
	No Sugar	Sugar "1"	Sugar "2"
50 min.	0	0	100
70 min.	0	0	70.2
80 min.	-	0	46.7
90 min.	-	0	25.0

Sugar "1": Free from all spoilage thermophiles

Sugar "2": High in spoilage thermophiles - especially thermophilic anaerobes

Only the more reactionary producers of refined sugars remained unconvinced, but with the canners' addiction to the purchase of only those

sugars that met the specifications of the National Canners Association, all refiners of sugars soon recognized that the manufacture of sugars for canning purposes had to be carried out with special care, in order to reduce thermophilic contamination.

The standards set by the National Canners Association were as follows:

1. Flat Sour Spores.	In the five samples examined there shall be a maximum of not more than seventy-five and an average of not more than fifty spores per 10 grams.
2. Thermophilic Anaerobic Spores.	These shall be present in not more than 60 percent of the five samples and in any one sample to the extent of not more than four of the six tubes.
3. Sulfide Spoilage Bacteria.	These shall be present in not more than two (40 per cent) of the five samples and in any one sample to the extent of not more than five spores per 10 grams. This would be equivalent to two colonies in the six inoculated tubes.

Incidentally, these standards were not arbitrarily selected but were derived from extensive tests of sugars having varying levels of thermophilic contamination. At the outset, some refiner claimed special manufacturing procedures for the production of sugars free from thermophilic bacteria, such as by the use of irradiation of the

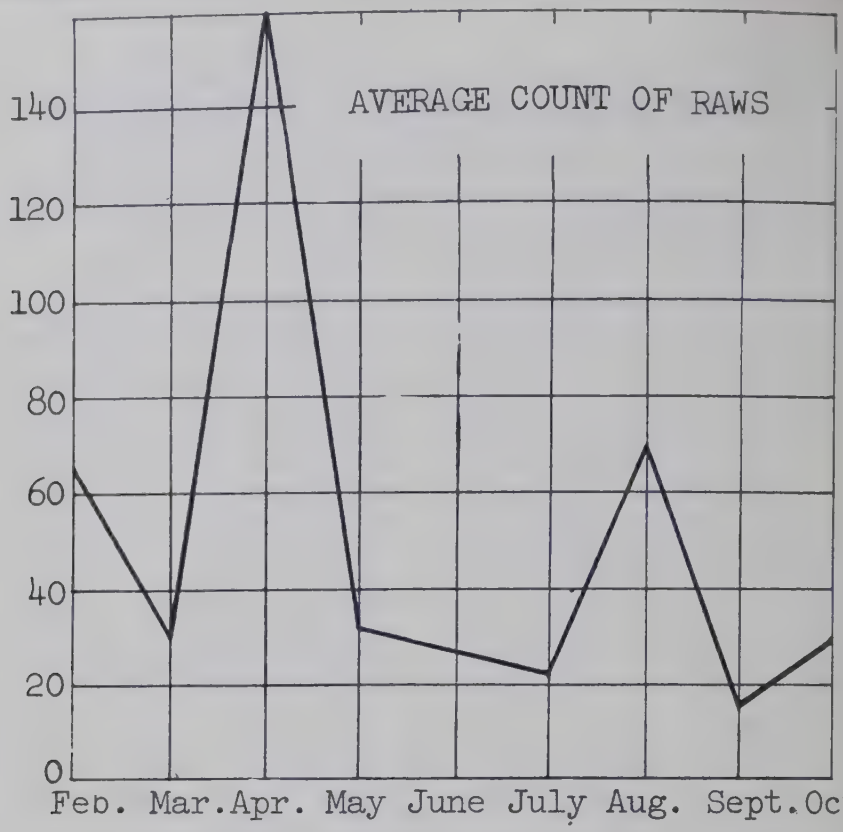
finished product or by special clarification procedures. It was soon realized, however, that the production of "canners' sugars" was one of selection and segregation, rather than of manufacture, for irrespective of the care used in refining, the thermophilic content of the finished product was entirely unpredictable. Hence, the accepted practice soon became one of selecting and segregating those lots of sugars that conformed to the National Standards, rather than attempting to vary procedures in the operations to achieve that end for the entire output of the refinery. We cannot enumerate, today, with any degree of assurance, all of the contributing factors to high thermophilic count sugars, although many are well known. It is assumed that these species originate principally in the soil although Owen (50) failed to find them in nine samples of soil examined. On the other hand, de Whalley and Scarr (51) found them to be abundant in soil samples, which they examined in the environment of a refinery. They are known to be present in large numbers in starch dust (52) and in dust samples collected in sugar refineries. Their growth in the steam bodies of the Effects and Vacuum Pans was noted by Calton (53) and Owen and Mobley (54) and Cameron and Williams (55) suggested that actual growth of these bacteria might occur in the stages of manufacture subsequent to filtration. That the thermophilic content of refined sugars does not consistently run parallel with the degree of this contamination in the raws being refined has been shown by Owen and Mobley (56) in their study over a period of years of the raws received and the sugar produced by a refinery. Their results are shown in the following table and the illustrative graphs reveal that there is a lag of thirty days between the high point of thermophilic contamination of the raws and that of the refined sugar produced from it and the high point of the latter

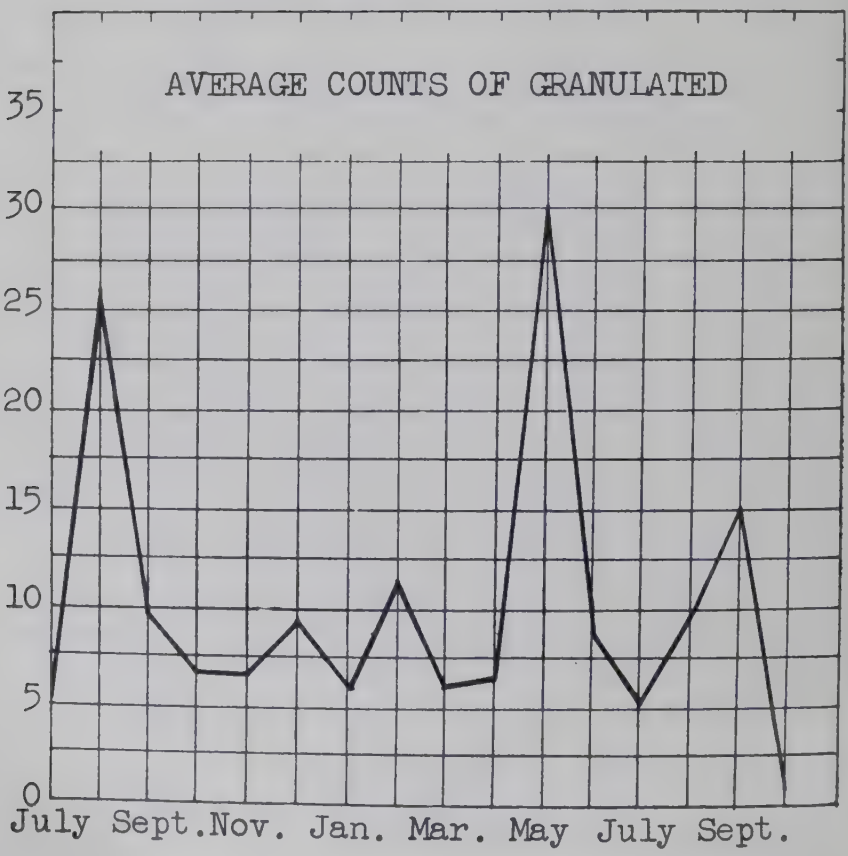
is in May and the low level of contamination is in October for this particular refinery.

Month	Average Thermophile Spores per 10 Grams	
	Raw Sugar	Granulated
March	80	5
April	325	2.5
May	160	7.5
June	32.5	2.5
July	27	7
August	22.5	7.5
September	70	30
October	16.6	5
November	53.7	8.7
December	25	8.3

These authors also noted (57) a consistent diurnal variation of the level of thermophilic contamination of the granulated sugar from this refinery, with the highest levels of contamination and incidence of occurrence in the afternoon sugars and the lowest at night. That the spores of thermophilic bacteria can survive the maximum temperatures to which sugar liquors are exposed, either in the raw sugar factory or the refinery, may be seen from the following results obtained by Cameron and Williams (58) on the death rate of this species: (See table on following page.)

Source and Number of Cultures	No. of Spores per [illegible].	Growth on Yeast Water Agar at 37° C. within 7 days	Minutes required to destroy spores at 110° C.	
			Inefficient	Efficient
Refined Cane Sugar	100,000	+		[illegible]
	6,000	+		[illegible]
12 Cultures	5,000,000	+	10	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	+	[illegible]	[illegible]
	100,000	-	120	130
	100,000	+	140	150
	100,000	-	220	240
Raw Cane Sugar	500	+		[illegible]
6 Cultures	100,000	+	15	[illegible]
	100,000	+	20	[illegible]
	100,000	+	25	30
	100,000	-	80	[illegible]
	100,000	-	200	220
Beet Sugar,	300,000	+		5
9 Cultures	10,000	+		10
	100,000	+		10
	100,000	-	15	20
	100,000	+	30	[illegible]
	100,000	+	50	[illegible]
	100,000	+	[illegible]	70
	100,000	+	80	[illegible]
	100,000	+	220	240


AVERAGE COUNT OF RAWS
140
120
100
80
60
40
20
0
Feb. Mar. Apr. May June July Aug. Sept. Oct.


AVERAGE COUNTS OF GRANULATED
35
30
25
20
15
10
5
0
July Sept. Nov. Jan. Mar. May July Sept.

THE SPECIES OF THERMOPHILIC BACTERIA IN SUGARS

While the three constituent groups of the thermophilic bacteria occurring in sugars have been rather extensively investigated, it is very probable that there are many variants from the recognized type species yet to be isolated. So far, the species identified with the three groups are as follows:

1. Flat Sours Spoilage. B. stearothermophilus (Donk (59))
2. Hydrogen Sulfide producing anaerobes. Clostridium nigrificands (Werkman & Weaver (60))
3. Hydrogen producing anaerobes. This group has received far less attention, as regards their classification, than the others, but we are indebted to Damon and Feirer (61) for their comprehensive study of these species isolated from manure. Their studies include the following species:

   Clos. thermoputrificum

   Clos. thermoaerogenes

   Clos. thermoacidophilus

   Clos. thermochainus

There is but little doubt that thermophilic characters are constantly being acquired by some of the mesophilic bacteria that are always present at all stages of sugar manufacture and one frequently observes so-called "spreader" colonies developing on plates of substrate chosen for flat sour analysis, when incubated at 55° C. These are non-acid producing species, but are typical in colony formation with the mesophilic

sporogenous species of soil bacteria. Their presence in sugars may be of more importance than is now realized, not on account of the ability to produce acids but because they may impart very unpleasant flavors to food or beverages.

THE POTENTIAL DETERIORATIVE ABILITY OF THERMOPHILIC BACTERIA UPON SUGAR FACTORY AND REFINERY LIQUORS

While there has been some evidence indicative of a definite role played by thermophilic bacteria in causing some of the undetermined losses of sucrose in sugar factories and refineries, there have been few controlled experiments to substantiate it. However, Cameron and Bigelow (62) did carry out some experimental work on this phase of the subject and concluded that the flat sour thermophiles could grow and produce acid in raw sugar solutions of 45 Brix density. We have noted in a previous portion of this work (cf. chapter on The Source of Microorganisms in Cane and Beet Juices) that McCleery (63) isolated from cane juices species of thermophilic bacteria that were capable of causing rapid losses of their sucrose content.

It is very probable that in some degree at least the development of these microorganisms in factories and refineries are responsible for the undetermined sucrose losses incident to normal operations. Except in those instances where large concentrations of viable thermophilic bacterial cells are detected in sugar liquor or cane juice, the amount of sucrose loss that can be charged to them is always difficult to determine, for to hold even a well buffered substrate at the optimum pH for the development of these microorganisms and for a sufficient period of time for the observation of their action would inevitably result in some

sucrose destruction. For the present we can indict them principally for the serious troubles which they are known to introduce in the canning industry, but it may be predicted that time will reveal more definite roles played by them in the losses of sucrose during raw sugar manufacture and its refining.

THE ELIMINATION OF THERMOPHILIC CONTAMINATION IN SUGAR REFINING

We have previously referred to the fact that present day methods of supplying to the canning industry sugars of low thermophilic contamination consist largely in the segregation of those lots of sugar that happen to meet the standards for that type of sugar, rather than by changing the methods of manufacture to attain that degree of purity in the finished product. Indeed, as far as the writer is aware, there is no guaranteed method of procedure of refining that has proven infallible in this connection, although some systems of clarification are more effective than others, even where the efficiencies are the same as measured by chemical criteria. On the other hand, the factors contributing to a high incidence of these contaminants in sugars are so well known that the most dangerous avenues of contamination can be easily avoided, but it might serve a good purpose to enumerate them in this chapter. We might well classify these avenues of contamination as

1. internal or autogenous,
2. external or exotic.

The first of these applies to the thermophilic contaminants that are introduced with the raw sugar and which are concentrated in some of the products and eliminated from others, or which in

some cases may be propagated in some of the liquors during the process of refining. As an illustration of how these bacteria are removed by filtration and unequally distributed between the finished sugar and the "run off" liquors, we cite the following results from the experiments of Cameron and Bigelow (64). In these experiments a syrup inoculated heavily with spores of flat sour bacteria was subjected to repeated filtrations, after being treated with a filter-aid at the rate of five pounds per 100 gallons of syrup.

REMOVAL OF FLAT SOUR SPORES BY FILTRATION AND CRYSTALLIZATION

Sample	Description	No. Flat Sour Spores per cc. of Syrup
1	Original inoculated syrup	25,000
2	First syrup through press	9,000
3	About 50 gals. (189.5 liters) through press	3,500
4	Average after all through first time	2,500
5	During second time through press	1,000
6	Average after all through second time	1,000
7	Average after all through third time	1,700
8	Average after all through fourth time	1,000
9	Composite 50 gals. (189.5 liters) sweet water	600
10	Mother liquor after centrifuge	2,200
11	Finished sugar	238*

*Spores per gram

It will be noted that the first filtration of the syrup reduced the level of viable cells from [illegible],000 to 9,000 per cc., but after four filtrations the number remaining in the syrup was 1,000 per cc. The finished sugar contained approximately 230 per gram, while the "run off" contained [illegible],200 per cc.

Owen and Mobley (65) reported similar results in their study of the thermophilic content of various intermediate liquors and the finished sugar in a refinery.

RESULTS OF EXAMINATION OF REFINERY SAMPLES
SET NO. 2

Location of Sample	Total Spore Count* 55° C.	Flat Sour Types 55° C.
Syrup entering filter press	6-9	0-1
Syrup leaving filter press	0-1 (in 6 gr. sug.)	0-1 (in 6 gr. sug.)
Char filter liquor-colorless	1 (in 6 gr. sug.)	1 (in 6 gr. sug.)
Char filter liquor-dark	100	100
Storage tank	80	[illegible]
No. 1 magma	280-370	[illegible]
No. 1 syrup from centrifugal	725	[illegible]
No. 1 sugar (wet)	5	[illegible]
No. 2 magma	650-700	[illegible]
No. 2 syrup from centrifugal	[illegible]	[illegible]
No. 2 sugar (wet)	[illegible]	[illegible]
No. 3 magma	[illegible]	[illegible]
No. 3 syrup from centrifugal	[illegible]	[illegible]

*Counts given as numbers of thermophilic spores per gram of sugar unless otherwise stated.

Here again, we note the high concentration of thermophilic spores in the "run offs" and in the massecuites. From the foregoing, we can now well appreciate the fact that the reduction of the autogenous thermophilic contamination in refined sugars depends upon

1. efficient clarification and filtration,
2. in the selection of first-strike sugars for the canning trade and
3. in the avoidance of holding intermediate dilute liquors within the temperature range for the development of thermophilic bacteria.

THE ELIMINATION OF EXTERNAL CONTAMINATION

The sources of external thermophilic contamination are as follows:

1. Dust from air
2. Starch dust from the starch used in manufacturing confectioners sugars
3. Tailings from the granulator.

We will now consider these sources of contamination in detail and will turn our attention first to air-borne contaminants. Owen and Mobley (66) investigated these sources of thermophilic contamination and subjected various refinery materials to analysis. The table on page 167 reveals the incidence of this contamination.

From this table, it will be noted that the tailings from the granulator are high in thermophilic bacterial count and there is no doubt that sugar dust and aggregates of crystals carry a large part of the thermophilic contamination that occurs in refined sugars.

Source of Sample	Flat Sour Spores	Anaerobes	
	per 10 grams	-H_2S	+H_2S
Standard Fine Granulated Going into Cooler	10	--	--
From Hopper	30	--	--
Dust from Granulated	10	--	--
Tailings from Standard Fine Granulated	70	--	--

The incidence of the contamination of the air around a refinery with thermophilic spores may be seen from the following data from the above authors' publication.

Source of Sample	Flat Sour Spores	Anaerobes	
	per 10 grams	-H_2S	+H_2S
Sample from Hopper	10	--	--
Dust Swept from Around Hopper	50	--	--
One Liter of air Around Hopper	137	--	--
Sterile Distilled Water Used in Aspiring Air Previous Test	0	--	--

Here, it will be seen that the air around the centrifugals in this particular refinery contained over one hundred flat sour spores per liter and as Church (67) has pointed out, the motion of the

centrifugals in purging sugars creates a tremendous air suction and exposes the sugar to the concentrated contamination incident to the contact with large volumes of air and its dust-borne contaminants.

It is well known that starch dust is highly contaminated, as a rule, with thermophilic bacteria and this is one of the principal sources of external contamination of a thermophilic nature. In their studies of the various sources of contamination of a sugar refinery, Owen and Mobley (68) obtained the following average counts from a large number of analyses. In these tests, 100 gram samples of a refined granulated sugar were exposed for several hours at various portions of the refinery and then analyzed for thermophilic bacteria. The results are shown in the following table:

FLAT SOUR THERMOPHILES IN EXPOSED SUGARS

Position Exposed	Flat Sours per 10 grams
Granulated stack	20.00
Raw-sugar dump	40.00
Starch mixer	230.00
Powder Mill	105.00
Warehouse scales	20.00
Sugar hopper	50.00
Centrifugals	45.00

More recently, de Whalley and Scarr (69) have reported an instance where a very high thermophilic contamination was acquired from Bone Char filters. In this instance, the authors report that the

contaminants brought into the plant with the raw sugars were almost completely eliminated by carbonation and filtration, but by subsequent passage of the filtered liquors through the Bone Black filters a very high incidence of thermophilic bacteria was acquired. Investigation disclosed that the source of this contamination was in dust in blind corners in the hoppers, under the kilns. An additional source of contamination was found in the cloths covering the false bottoms at the base of the Char filters and only by subjecting these to sterilization under steam pressure was the infection eliminated. From this interesting and significant observation it would appear that in many instances thermophilic destruction of sucrose in Char filters might be a distinct possibility and that some of the undetermined losses in refining might be attributed thereto.

THE COMPOSITION OF REFINED SUGARS AS EFFECTING MICROBIAL GROWTH IN SUBSTRATES MADE FROM THEM

There is still another appraisal of refined sugars based upon microbiological criteria, which is of growing importance in the industries using these products, and we refer to the qualities of the impurities of the product which stimulate or promote the development of microorganisms. This is a very essential criterion in the selection of sugars used for certain industries, such as carbonated beverages and certain food products, for experience has shown that even the traces of impurities carried by certain sugars of apparently the highest purity may greatly stimulate the growth of yeast and other microorganisms in beverages and foods that are not subjected to sterilization. This observation is by no means recent, for Pasteur (70), as early as 1860, had observed differences in the rate of growth of yeast in substrates prepared from various lots of sugar in

conjunction with a standard mineral enrichment formula. Windisch (71), Amand (72) and Kossowi (73) also contributed convincing data on the variability of sugars of high purity, as regard their "growth promoting" properties in fermenta tion. As early as 1902, Wildiers (74) had iso- lated a so-called "bios", or growth promoting principle, which he regarded as essential for microbial growth, and the final proof of the ex istence of definite chemical entities constitut such essential growth principles was establishe by the brilliant work of Funk and Freedman (75) in 1923. These latter investigators adduced va proof that refined sugars carried definite grow promoting substances in varying and entirely un predictable amounts. The nature of these growt stimulating complexes has been extensively re- viewed by Tanner (76), Miller (77) and Peskett (78). More recently, Williams (79) compiled a comprehensive review of the subject and from th latest contributions it has been definitely de- termined that "yeast biotin" is a member of the Vitamin B complex. The presence of this vitami in refined sugars was first reported by Cheldel and Williams (80) in 1942 and the amounts found be present were less than 0.004 micrograms per gram of the product examined.

Hall, James and Stuart (81) were the first investigators to subject this aspect of the com sition of refined or direct consumption sugar t an extensive investigation, with the view of appraising the risk to industrial users of this commodity, as regards the spoilage by fermentat of the products in which it is used and which, like beverages and certain foods, are not subje ed either to pasteurization or sterilization temperatures. In their investigations, the pla of procedure followed was to make 10 per cent solutions of refined granulated sugars and afte sterilizing them, each sample was seeded with

nt at the rate which would bring the yeast coun
the test solution up to approximately 50,000
ls per Cc. Their results are shown in the
lowing table.

r	Number of Samples	Number of cells inoculated per ml.	Yeast-inoculum multiples		
			Average	Minimum	Maximum
1	29	35,000	6.6	1.0	23.0
2	68	47,000	8.5	1.0	80.8*
3	38	50,000	6.9	1.0	30.4
4	39	48,000	4.8	1.0	13.4
5	65	49,000	12.4	1.0	59.6
6	70	47,000	8.6	1.1	27.4
7	70	51,000	8.0	2.6	19.2
8	77	50,000	7.6	1.4	25.2**
9	75	53,400	9.1	1.9	20.2***
0	87	52,000	8.0	1.1	18.4
1	87	50,000	6.6	1.0	16.6
2	87	51,000	4.2	1.0	18.8

*Only one sample above 39.2

**Only one sample above 18.2

***Only one sample above 24.2

Hall, H. H., James, L.H. & Stuart, L. S. Yeast Growth-Promoting Substances in White Sugar. Food Research, Vol. 12, No. 2, March-April, 1947.

From the above results, it will be noted that variations in the amounts of "growth promoting"

substances from year to year are extremely large and that the sugar from the same factories may show more than twice as much of these substances one year than they do another. Incidentally, these investigators had observed that in their procedure C. P. sucrose solutions prepared in an identical fashion as their test solutions supported no growth of yeast whatever. Recently, Hall, Paine and Fabian (82) have subjected this subject to a more intensive investigation, in an attempt to correlate the "growth promoting" properties of refined sugars with the ash constituents of the sugars in which they occur. The following table is taken from their published report of their extensive investigations on this subject.

Year	Average for all samples		Ash Average for group		No. of samples in group	
	Yeast Inoculum multiple	Ash*	With yeast inoculum multiple below average	With yeast inoculum multiple above average	With yeast inoculum multiple below average	With yeast inoculum multiple above average
		pct.	pct.	pct.		
1931	6.6	.0274	.0244	.0348	19	10
1932	8.5	.0245	.0219	.0246	50	18
1933	6.9	.0197	.0186	.0222	40	18
1934	4.8	.0140	.0108	.0169	36	23
1935	12.4	.0132	.0116	.0156	47	18
1936	8.6	.0135	.0130	.0151	38	32
1937	8.0	.0116	.0105	.0151	37	33
1938	7.6	.0109	.0106	.0116	47	30
1939	9.1	.0110	.0104	.0117	37	38
1940	8.0	.0099	.0095	.0104	45	42
1941	8.6	.0091	.0082	.0101	49	38
1942	4.9	.0111	.0140	.0113	53	34

Hall, H. H., Paine, H. S. and Fabian, F. W. Yeast Growth-Promoting Substances in White Sugar. Food Research. Vol. 12, No. 2, March-April, 1947.

*The ash determinations were made by Sam Byall, Chemist, Bureau of Agriculture and Industrial Chemistry, as part of a study of sugar impurities.

It will be noted in the above table that there is a correlation between the ash and the presence of yeast-growth stimulants in the samples examined and that the average ash contents of the samples, showing yeast multiplication, were consistently higher than those which did not support yeast growth. One of the most illuminating disclosures of the investigations by the above authors is to be found in their study of the comparison between the comparative amounts of these yeast-growth stimulants in the first massecuites and the sugars made from them, although the authors commented principally upon the lack of agreement between individual values in the various samples subjected to analysis. While consistency was indeed lacking, the evidence is entirely convincing that the "run off" at the centrifugals rather than the sugar crystals themselves retains most of these "growth promoting" entities. Their published results are shown in the following table.

Steffen Yeast-inoculum multiple			Non-Steffen Yeast inoculum multiple		
Factory	Massecuites	Sugar	Factory	Massecuites	Sugar
A	92	10.0	A	168	8.6
B	154	10.6	B	272	8.6
C	156	8.0	C	322	5.6
D	162	9.8	D	326	6.6
E	180	6.6	E	354	10.4
F	198	4.2	F	356	4.8
G	214	16.6	G	360	4.8
H	230	3.8	H	411	4.4
I	232	6.3	I	452	10.2
J	260	9.0	J	454	3.2
K	330	3.6	K	526	3.6
L	332	8.0	L	660	11.4
M	378	5.0	M	842	6.8
N	414	12.2	...	...	....

The comparative amounts of "biotin" in refined cane and beet sugars were also studied by these same investigators and their results are given below.

Sample No.	Type of sugar	Biotin (γmg. per gram)	Yeast inoculum multiple
C-1	Cane	.445	7.8
C-4	Cane	.390	7.2
C-3	Cane	.075	6.1
4166	Beet	.060	3.7
C-5	Cane	.055	14.5
4165	Beet	.040	5.5
C-2	Cane	.035	3.2
4111	Beet	.025	2.9

It is interesting to observe that while the cane sugars analyzed and reported in the above table have a much higher "biotin" content than the beet samples, the factor of growth stimulation was by no means parallel with this difference. There was one instance, however, where a cane sugar gave a very large increase in yeast growth, in fact the largest reported, while showing a "biotin" assay little above the average for the best products.

The limits of "biotin" found in refined cane and beet sugar samples, which they examined, varied from 0.025 to 0.445 micromilligrams per gram of sugar and when these values are compared with the minimum requirements of this growth factor for yeast growth it can be well appreciated, the critical role high "biotin" values in refined

sugars might play in contributing to the spoilage of sugar solutions and foods in which they form a principal constituent. For example, Kogl and Fries (83), in their investigations, found that 0.00004 micrograms of "biotin" is required per two cc's of substrate to sustain a 100 percent increase in yeast cells. Calculated to micromilligrams, this would be 0.02 per cc. Hence, a sugar solution of 10 percent concentration prepared from a sugar having a concentration of 0.445 per gram would more than sustain this increase in yeast growth. The importance of this phase of sugar microbiology is being appreciated more and more keenly in the widening industrial usages of sugar for beverages and for unsterilized food products. On one occasion, the writer (84) had to resort to applying a pre-fermentation procedure to a beverage concentration, consisting of sugar syrups and flavoring material, in order to eliminate its "growth promoting" factors. By fermenting the syrup with yeast and filtering off the cells, the spoilage problem was entirely solved and the distinctive flavor of the beverage was enhanced, rather than impaired, by the process.

The quality of refined sugars today, with respect to the minute impurities which even those of the highest chemical purity may harbor, is a subject of growing importance, for in spite of the fact that refined sugar is one of the purest chemicals produced on an industrial scale the high concentrations in which it is used in sweetened foods and beverages make even these minute impurities of great importance.

However, it is in the rapidly expanding field of liquid sugar manufacture that these "growth factors" become critically important, for as we have pointed out in a previous portion of this publication the prevention of deterioration of these products during room temperature storage and

exposures to atmospheric temperatures, while in transit, depends largely upon the degree to which they are contaminated with yeast, but also upon their chemical characteristics as regards the presence of trace impurities. Both the kind and amounts of ash affect their susceptibility to fermentation, but as noted in the citations from the published data on this subject the concentration of these minute amounts of "bioses" is usually higher in the liquid portions of the massecuites than in the crystals separated from them. Crystallization would be expected to purify the crystals at the expense of the mother liquor and hence special procedures, as regards clarification and filtration, may be required to produce a liquid sugar in which "bioses" concentration would not exceed the levels attained in a granulated, refined sugar, produced from the same quality of raws. The fact that this liquid product is as stable as it has proven to be reflects the care and thoroughness of chemical control used in its manufacture.

METHODS FOR THE DETERMINATION OF MICROORGANISMS IN RAW AND REFINED SUGARS

The microbiological appraisal of raw sugars is usually confined to the quantitative determination of mold fungi, or to thermophilic bacteria, and in rare instances to the viable yeasts that these sugars may contain. The interest in the microbial flora of these products is usually confined to the effect of this flora and its concentration upon the keeping qualities of the sugar and since the mold fungi are the principal deteriorative agents occurring in these products a determination of their presence and concentration usually fulfills all of the requirements that may arise. Sometimes it becomes expedient for the refiner to trace outbreaks of excessive thermophili

contaminations in his finished products to their original sources and in such cases a thermophilic assay of the raws entering the plant is indicated. Unfortunately, as has been previously pointed out, there seems to be no consistent correlation between the degree of thermophilic contamination of raws and the refined sugars produced from them. However, the growing suspicion in the minds of the sugar refinery technicians that some considerable part of the undetermined losses of sucrose during raw sugar manufacture is the result of the action of thermophilic bacteria will probably lead to a more extensive appraisal of the numbers of these microorganisms occurring in the intermediate stages of raw sugar manufacture.

The determination of mesophilic bacteria in raw sugars may also have more significance than is at present believed, for if these are, as we now suspect, principally sporogenous species and represent the survival of the bacteria occurring in the extracted cane juice then their concentration in the finished sugar might be taken as an index of the efficiency of clarification or the level of sanitation in the factory in which the sugar is produced.

At times, a qualitative analysis of raw sugars for the detection of specific types of mesophilic bacteria may be of significance, as for example, to ascertain the presence of gum-producing bacteria, such as the levan formers and in rare instances for the presence of Leuconostoc mesenteroides, the species forming dextran.

While it may be far in the future before all of these determinations are applied as routine procedure in raw sugar factories, they are essential in the investigation of the role of raw and refined sugars as carriers of food spoilage microorganisms and the results obtained from them are

of more than theoretical interest in acquiring a knowledge of the capacity of the vegetative forms of bacteria to survive in substrates of low moisture and high osmotic levels.

THE DETERMINATION OF MOLD FUNGI IN RAW SUGARS

For these assays, the substrates recommended are as follows:

1. Kopeloff Agar (modification of Czapek's Agar) (85)

Cane Sugar	50.00 grams
Peptone	5.00 "
K_2HOP_4	1.00 "
KCl	0.50 "
$MgSO_4$	0.25 "
$FeSO_4$	0.01 "
NH_4NO_3	1.00 "
Agar agar	20.00 "
Water	1,000.00 cc's

2. Browne's Raw Sugar Agar (86)

A 30 per cent solution of 96-test sugar is boiled with a little salt-free alumina cream, filtered and diluted to 20 Brix. To this solution agar agar is added in the proportion of 15 grams per liter.

3. Owen's modification of Smith's Agar (87)

Cane Sugar	400 grams
Potassium Chloride	5.0 grams
Sodium Phosphate	2.0 "
Peptone	1.0 "
Agar Agar	20.0 "
Water	1,000.0 cc's.

The advantage in using this high density substrate is that it limits the growth of all fungi except those that are of significance in inducing the deterioration of crystal sugar.

There are a number of formulae for substrates particularly well adapted to the development of mold fungi, but these are designed to exclude other groups by virtue of the attainment of a low pH, by the addition of acid. These procedures are not well adapted to the present purposes, as they promote rather than inhibit the growth of irrelevant species of mold fungi. Substrates of this category are the following:

4. Malt Agar (Reddish (88))

Malt extract	30 grams
Agar Agar	15 "
Water	1,000 cc's

Acidified with lactic acid to a pH of 3.5 just before pouring. Prescribed amount of acid for adjustment 2.00 cc's of a 10 percent solution of U. S. P., 85 percent lactic acid per 100 cc's of substrate.

5. Sabouraud's agar (89)

Bacto Peptone	10 grams
Bacto Dextrose	40 grams
Bacto Agar	15 grams
Water	1,000 cc's

For the suppression of bacteria, T. L. Chin suggests the addition of 0.015 percent of potassium tellurite or 0.05 percent of copper sulphate.

Procedure for the Determination of Mold Fungi in Sugars

The dilutions of sample required for the assay of mold fungi in sugars are, as a rule, from 1:10

to 1:100. The water blanks are prepared by measuring out 100 cc's of water in bottles or flasks of 200 cc's capacity and sterilizing at 15 pounds steam pressure for 30 minutes. If bottles are used, rubber stoppers are used for closures, and if flasks are employed, cotton plugs may be used for the purpose. Ten grams of the sugar under examination are weighed into the water blanks and the sugar dissolved by shaking and one cc. portions introduced into sterile plates. If 1:100 dilutions are required, 10 cc's of the 1:10 dilutions are introduced into a second water blank and one cc. of this transferred by sterile pipettes into the culture dishes.

An incubation temperature of from 30 to 34 degrees C. is desirable for these analyses and a period of incubation of at least 72 hours.

The Microscopical Examination of Raw Sugars for Mold Fungi

In many cases, excessive mold concentration in raw sugar samples may be detected by a direct microscopical examination. Of course, this procedure does not differentiate between viable and non-viable mold spores and mycelia, but at least it gives a fairly adequate picture of the growth that has taken place and the extent of the contamination with mold fungi. For this purpose, it is desirable to wash off the molasses films surrounding the sugar crystals with ethyl alcohol of 96 percent strength, which will dissolve the film but leave the sugar crystals intact. Ten grams of the sugar to be examined are weighed into a fifty cc. Erlenmeyer Flask or in a 50 cc. test tube. Ten or twenty cc's of the alcohol is then added and the crystals vigorously shaken for several minutes. A drop of the liquid is then transferred to a slide and examined under the microscope, or if records are to be kept as to the concentration

of mold spores and portions of mold mycelia in the samples examined, a Haemycytomer may be employed or a Howard Cell Counter.

The Detection of and Enumeration of Mesophilic Bacteria in Sugars

The only group of mesophilic bacteria normally occurring in sugars are the spores of the mesenteric group of levan-forming species. While these are constantly present in both raw and refined sugars, they rarely, if ever, are responsible for any deteriorative changes in either product. It is important at times to determine their numbers, as their levels of concentration may indicate the efficiency of clarification procedures and the amount of dust-borne contamination in the factory or refinery in which the sugars are produced. The best substrate to use for this purpose is the following:

Owen's Modification of Grieg Smith's Agar - 50% Sugar Concentration

The formula for this medium has already been given under the methods for the determination of the mold fungi, the only difference being in the higher concentration of sugar used in its preparation.

The procedure for making the dilutions is the same as previously described, as the dilutions are from 1:10 and 1:100.

The colonies formed by these gum-producing bacteria are easily identified, as they are usually translucent and viscous and frequently spread over relatively large areas of the surface of the plates.

The Detection and Enumeration of Yeasts in Sugars

The substrates given in connection with the methods of analyses of syrups, liquid sugars and molasses apply so well to the present purpose that we need not repeat them. The procedure recommended in that connection is also applicable, as the dilutions required by the former products in their incipient stages of deterioration will cover the situation presented by the average raw or refined sugar. The only difference is in the fact that in the sugar assay the sample has to be weighed into the dilution bottle, while syrups can be pipetted, although even there it is preferable to weigh the sample rather than to operate on a volume basis.

THE DETERMINATION OF THE THERMOPHILIC BACTERIA IN REFINED SUGARS

Since all of the procedures given in the foregoing discussion of methods of determining the microorganisms in raw sugars are applicable to refined, as well, we need only concern ourselves, at this time, with the thermophilic assay.

Before giving the methods of procedure, it might be appropriate to call attention to the unusually large sources of error inherent in these appraisals. The factors of errors inherent in these analyses may be classified as follows:

1. The minute concentrations in which these bacteria occur in the products under examination.
2. The unequal distribution of the contaminants in the product.
3. The probable inhibiting action of non-significant species of bacteria upon the ones that are being appraised.

Considering the first of these factors, it may be said that in no food product and in few therapeutic preparations is the presence of as few non-pathogenic bacteria regarded as of having any significance whatever. Since the standard limits the number of "flat sours" bacteria to 50 spores per 10 grams of sugar, this means a count of only five per gram. It is difficult to conceive of uniform distribution of such minute amounts of matter in such a relatively large mass of material. One may conceivably take 10 samples of 20 grams each from a half pound of sugar and obtain widely different values, depending upon in what portion of the original lot the bacterial spores were concentrated. If, instead of viewing the dispersal from the point of view of the number of cells distributed in so many grams of sugar, we take the ratio of the respective masses of the spores and the sample examined, the chances of uniformity in distribution becomes astronomically remote.

2. The unequal distribution of the bacteria in the sugar -

In the light of our present knowledge of the source of this contamination and its variety, embracing dust from the air and some of autogenous origin, and the tendency of the contaminants to become concentrated in the liquors and ejected from the crystal sugar, we can safely assume that false grains of sugar or aggregates of crystals will carry a far greater concentration of spores than will be found in the normal free-running crystals. Also, sugar dust will likely carry an abnormally higher concentration than do the crystals and both of these exotic forms of sugar will inevitably be very unequally dispersed through the mass of crystal sugar.

3. The probable inhibiting action of the non-significant species of thermophilic bacteria upon the ones that are being appraised -

In the present standards, all bacterial colonies occurring on the plates seeded for the "flat sour" counts are included in the total count for thermophiles. The limits tolerated for this total count are 150 spores per ten grams, while the limits for the "flat sour" are 50 spores for the same weight of sample. Owen and Mobley (90), in their investigations of the thermophilic flora of refined sugars, found that there seemed to be an inverse ratio between the levels of total thermophilic count on plates and counts for "flat sours". That is to say, with a high total count of non-acid forming thermophilic aerobes the acid-producing types, or "flat sours" counts, were almost invariably low. The fact that many of the non-acid thermophilic aerobes are related to those species of bacteria occurring normally in soils and which produce antibiotic substances, would infer that the development of colonies of these species might inhibit the development of the "flat sours" types. Waksman (91), for example, claims that B. mesentericus produces a toxin substance very similar in its antibiotic effects as to pyocyanase.

Many of these species, which are classified as mesophilic in their temperature relations, have a maximum level of from 50 - 55 degrees C.

METHODS OF NATIONAL CANNERS ASSOCIATION FOR THE DETERMINATION OF THE NUMBERS OF THERMOPHILIC BACTERIA IN REFINED SUGARS

The following procedures will be used where a member canner buys sugar on specification and requests that this laboratory determine whether the specifications are met.

SAMPLING -

"One-half pound samples will be taken from each of five bags or barrels of the shipment or of the lot in question. These samples will be sent to the laboratory in clean sealed cans, or other appropriate containers.[1]

PREPARATION OF SAMPLE -

"Place 20 grams of sugar in a sterile 150 cc. Erlenmeyer flask marked to indicate a volume of 100 cc. Add sterile water to the 100 cc. mark. Bring rapidly to boiling and boil for five minutes. Replace evaporation with sterile water.

DETECTION OF FLAT SOUR SPORES -

"Into each of five petri dishes, pipette 2 cc's of the boiled sugar solution. Cover and mix the inoculum with Bacto dextrose tryptone agar.[2] Incubate the plates at 55° C. for 36 to 48 hours. In order to prevent drying of the agar, the incubator should be humidified. The combined count from the five plates represents the number of spores in two grams of the original sugar. Multiply this count by five, in order to express results in terms of number of spores per 10 grams of sugar.

"Flat sour colonies are characteristic. The colony is round, measures from two to five millimeters in diameter, presents a typical opaque central "spot", and by reason of acid production in the presence of the indicator, is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant, or missing, where certain low acid-producing types are concerned, or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical

subsurface colonies are rather compact and may approach the "pinpoint" condition.

"Where there is doubt as to the identity of the sub-surface colonies, a decision can usually be made by observing the nature of the surface colonies. Where the surface colonies evidence reasonable purity of flora, it is safe for practical purposes to assume that the sub-surface colonies have been formed by similar bacterial groups. It is emphasized that where the plate is heavily seeded, there may be loss of accuracy as regards counts and colony structure and size may be atypical. Where plates are so heavily seeded as to make counting impracticable, a second sample of the sugar may be plated, using dilutions of the original solution. For practical purposes, however, it is sufficient to note that the sample is obviously below standard.

"At times, the nature of sub-surface colonies is in question. Whether they are flat sour colonies may often be determined by transferring by the stroke method, from the colonies to agar plates. Their surface characteristics may then be noted.

TOTAL COUNT -

"The total thermophilic spore count is made from the same set of petri dishes. Calculation of the count is made in the same way as noted above for flat sour spores.

DETECTION OF THERMOPHILIC ANAEROBES NOT PRODUCING H_2S -

"Divide 20 cc's of the sugar solution approximately equally among six freshly exhausted liver broth[3] tubes and stratify the liquid medium with

plain nutrient agar. After the agar has solidified, preheat to 55° C. and incubate at that temperature for 48 hours.

"Under the conditions stated, thermophilic anaerobes are manifest through the splitting of agar and the presence of acid. At times a "cheesy" odor is noted. The method is considered suitable as a qualitative test but quantitatively it provides only a means for estimation. The method does not permit expression of results in terms of numbers of spores per unit weight of sugar.

DETECTION OF THERMOPHILIC ANAEROBES PRODUCING H_2S (sulfide spoilage) -

"This group is made up of the so-called sulfide spoilage organisms. Divide 20 cc's of the sugar solution approximately equally among six tubes containing "sulfite" agar.[4] Make inoculations in freshly exhausted deep agar tubes. Incubate at 55° C. for 48 hours.

"In "sulfite" agar the "sulfide" spoilage organisms are detected through the formation of characteristic blackened spherical areas. Due to the solubility of hydrogen sulfide and its fixation by the iron, no gas is noted. Certain of the thermophilic anaerobes (not producing H_2S), methods for the detection of which have been given above, give rise to relatively large amounts of hydrogen which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.

REPORTING RESULTS -

"Report total counts and flat sour and sulfide spoilage results as number of spores per 10 grams

of sugar. Report thermophilic anaerobes (not producing H_2S), as number of tubes positive and number negative in the following manner: + + +, - - -.

NATIONAL CANNERS ASSOCIATION STANDARDS

Total Thermophilic Spore Count:

"For the five samples examined, there shall be a maximum of not more than 150 spores and an average of not more than 125 spores per 10 grams of sugars.

Flat Sour Spores:

"For the five samples examined, there shall be a maximum of not more than 75 spores and an average of not more than 50 spores per 10 grams of sugar.

Thermophilic Anaerobic Spores:

"These shall be present in not more than three (60 percent) of the five samples and in any one sample to the extent of not more than four (65 + percent) tubes.

Sulfide Spoilage Spores:

"These shall be present in not more than two (40 percent) of the five samples and in any one sample to the extent of not more than five spores per 10 grams. This would be equivalent to two colonies in the six inoculated tubes.

NOTES

1. "It is appreciated that the adequacy of this sampling will vary in relation to the size of

the shipment or lot, but it is felt that where there is any significant variability in the shipment this fact will become evident in the majority of cases, through individual tests on five samples.

2. "This medium may be obtained in dehydrated condition from the Difco Laboratories, Detroit Michigan, or from the various supply houses that carry Difco products in stock. Bacto Dextrose Tryptone agar was developed by the Difco Laboratories, in collaboration with this laboratory with this special purpose in mind. It appears peculiarly adapted to growth of flat sour bacteria and in the medium the colonies, both surface and sub-surface, exhibit distinctive growth. Sub-surface colonies are larger than in media formerly suggested. Further advantages lie in its standardization and convenience in preparation.

"Some lots of Brom-cresol-purple indicator carry a bacteriostatic factor. It is suggested, therefore, that those laboratories which prepare their own medium from ingredients test each lot of this indicator for suitability with a sugar of known contamination.

3. "500 grams of chopped beef liver are mixed with 1000 cc's of water and boiled slowly for one hour, after which the boiled material is pressed through cheese cloth and the liquid is made to 1000 cc's. To the broth are added 10 grams peptone and one gram K_2HPO_4. The reaction is adjusted to pH 7.0. In tubing, one-half inch to one inch of the previously boiled ground liver is introduced into the tube.

4. "The sulfite agar previously recommended has been superseded by the following medium, which is more easily prepared and which has been found to be fully satisfactory:

Water	1000 ml.
Tryptone	10 gms.
Sodium sulfite	1 gm.
Agar	20 gms.

No adjustment of reaction is necessary

"At the time of tubing, a clean iron strip or nail is placed in the tube and covered with the medium."

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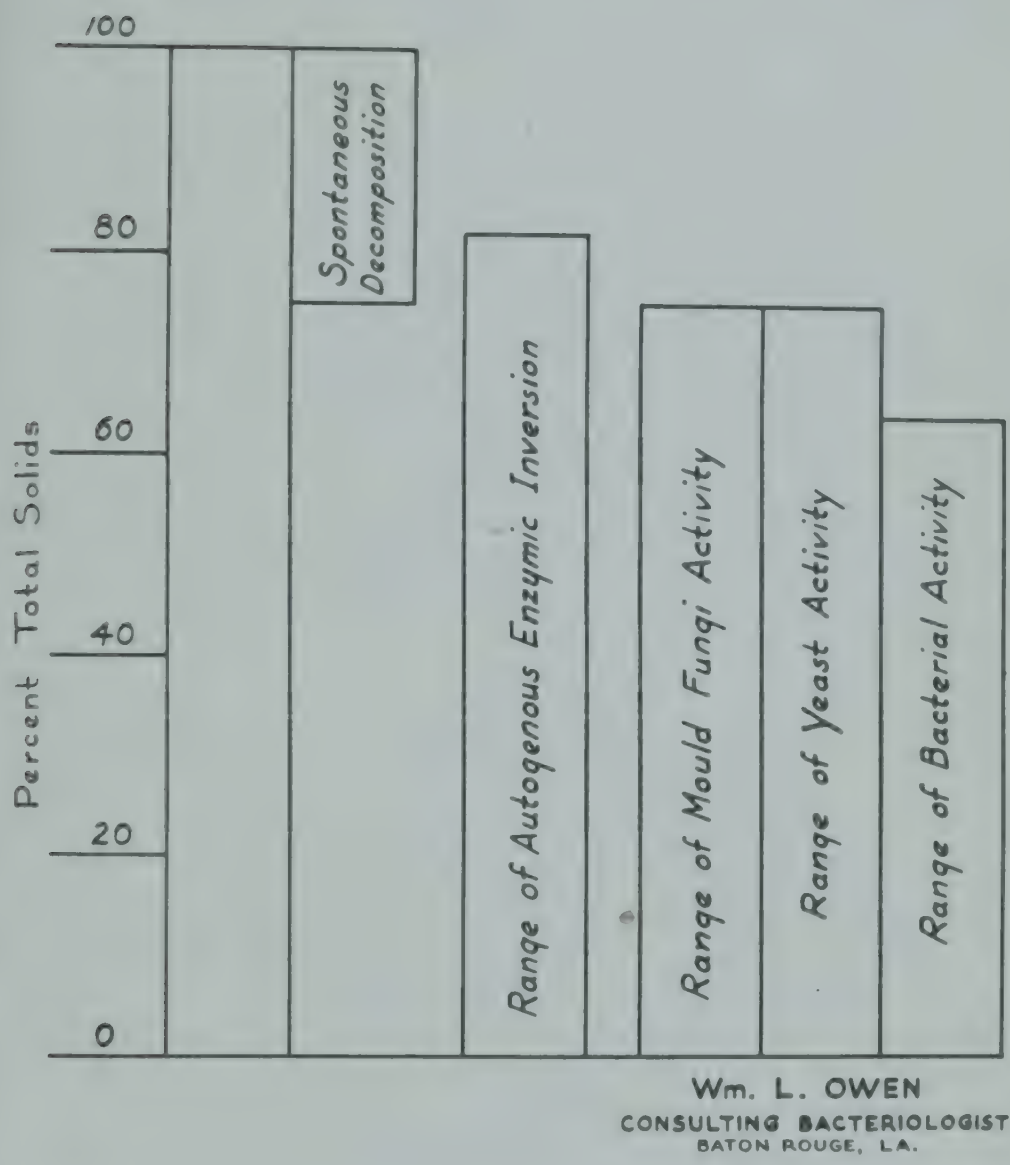
Chapter IX

DETERIORATION OF BLACKSTRAP MOLASSES IN STORAGE

While this chapter might seem quite alien to the scope of the present volume, since it is not a microbiological, but a chemical, phenomenon, its inclusion seems warranted, if for no other reason than to dispel, if possible, any doubts that may exist, regarding its purely chemical status and to exonerate microorganisms of any responsibility for it. It should be stated, however, that from an economic standpoint it is becoming increasingly important and the losses from this cause to the producer of this commodity, which up until comparatively recently was quite rare, is now becoming quite serious and blackstrap of normal composition and density which was formerly regarded as being entirely stable, under suitable storage conditions, although the distiller often complained that old stocks of material seldom gave fermentation efficiencies comparable to new crop molasses, the sugar values tended to remain fairly constant. The lower yields of alcohol from the old, as compared with the fresh material, seemed indicative of a slow change in the fermentability of the sugars that it contained. These observations on both the loss of sugars in blackstraps, during storage, and the depreciation in the fermentability values of the sugars that remain have been recorded in the literature on the subject over a period of at least two decades. Owen (1) gave a partial review of the literature, as relating to the possible role of microorganisms as causative agents of it. In that review, the work of Browne (2), Geerligs (3), Kopfler (4), Habif (5) and Hucker and Pederson (6)

cited and the status of the subject as of that time (1944) carefully appraised. It was concluded from that study that at normal densities blackstrap is practically immune from microbial action. It is not improbable that on the surface of molasses, exposed to the atmosphere, there may be zones of low density, in which mold fungi and yeast become active, but these areas are so small in proportion to the entire mass that the results of microbial activity are undetectable by analysis. In Hot Rooms of sugar factories, one may often note profuse growths of mold fungi on the surface of massecuites and yet no decrease in the purity of the product has ever been attributed to this cause. On the contrary, many factory superintendents are of the opinion that massecuites so effected are more readily "purged" than are those which are free from it. This concept may have some validity, in that it may be correlated with an improved physical condition of the massecuites preceding the appearance of the mold growth and responsible for the latter rather than a result of the mold growth. Hucker and Brooks (7) have in recent years subjected the phenomenon of the gas evolution of molasses to an exhaustive investigation, with the view of determining its causes and the conditions contributing, thereto. While they found in frothing molasses there was to be expected many species of microorganisms that could produce gas from hexose sugars in substrates of low densities, they failed to acquire any conclusive evidence of the microbial origin of this evolved gas in foaming molasses. The writer (8) gave an appraisal of the maximum density limits at which any of the groups of microorganisms known to occur in blackstraps could induce its deterioration and concluded that this extreme upper limit was in the range of 75 to 80 Brix. (See chart on following page.)

CHART SHOWING DENSITY LIMITS OF MICROBIAL AND CHEMICAL ACTION IN MOLASSES DURING STORAGE



Courtesy of SUGAR, N. Y. Publishers of "Motor Fuel from Molasses".

As far as the writer is aware, an authentic case is yet to be reported, where the quantitative microbial analysis has revealed levels of population density that could be regarded as commensurate with perceptible destruction of sugars in a blackstrap molasses of normal density. We will have occasion to discuss this lack of correlation later on, in connection with cases of deterioration of blackstraps that we have studied.

THE RATE OF DETERIORATION OF BLACKSTRAPS IN STORAGE

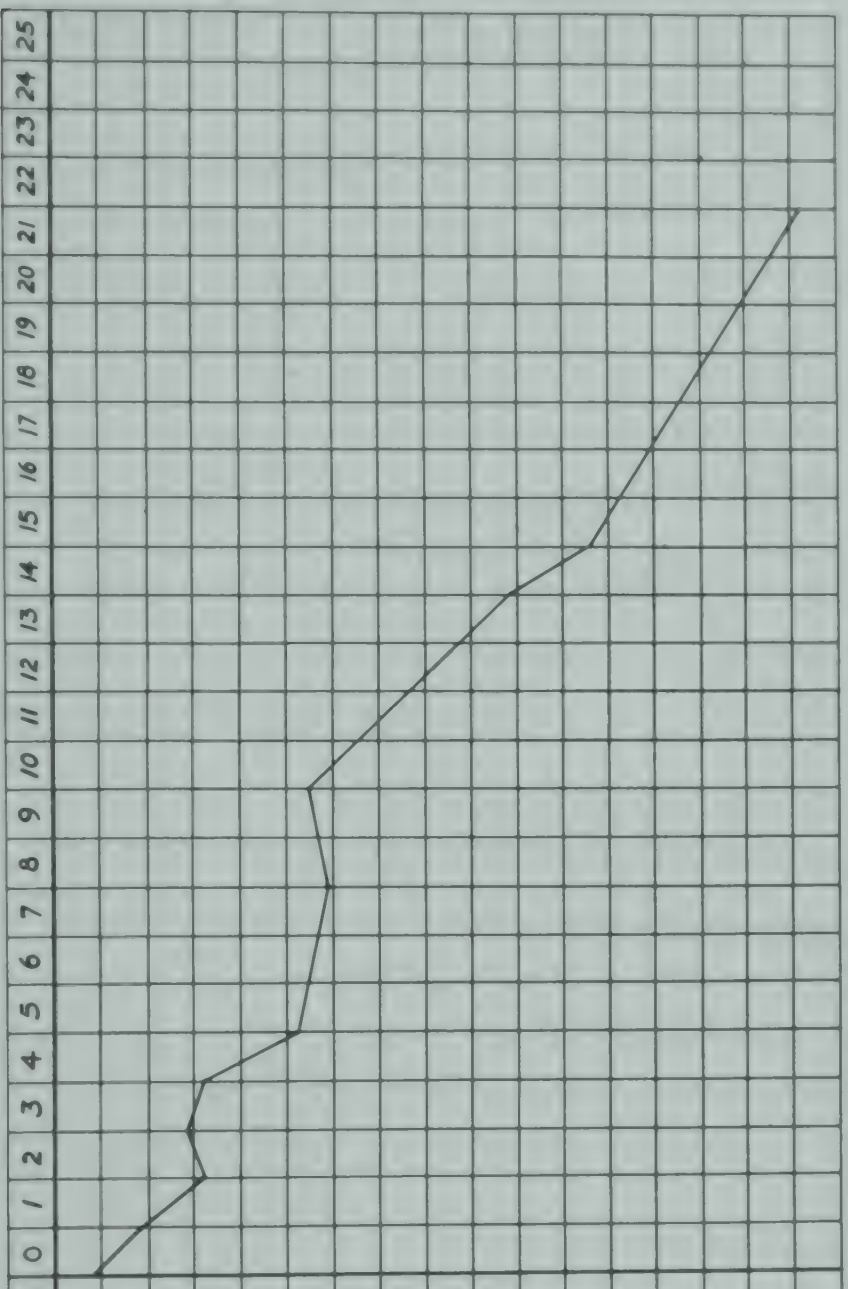
The concept which we have previously entertained, regarding this phenomenon, is that it is very slow and that months and even years are required to reveal any perceptible change in analyses of blackstraps in storage. The classic observations of Browne (9) and of Geerligs (10) indicate the slow rate of change that these products undergo. The following tables and charts from the publication by Browne (11) show the deterioration of a sample of blackstrap over a period of twenty years.

Date	Polarization	Sucrose	Invert	Total Sugar as Invert
April, 1914	24.86	31.30	19.10	52.04
Oct., 1914	23.54	30.73	18.74	51.09
May, 1915	22.33	30.00	18.19	51.09
April, 1916	21.34	29.74	18.84	50.14
Sept., 1917	19.80	28.45	19.93	49.88
Aug., 1918	18.26	25.81	20.66	47.83
Aug., 1921	.....	24.93	20.93	47.17
Jan., 1923	.....	25.13	21.07	47.52
July, 1927	11.84	20.98	21.00	43.08
March, 1928	.....	18.94	21.50	41.44
March, 1935	6.40	12.61	23.57	36.84
Loss in total sugars 15.20 Pct.				

(Chart on following page.)

CHART SHOWING DECREASE IN TOTAL SUGARS IN MOLASSES DURING STORAGE

CALCULATED FROM BROWNE'S DATA



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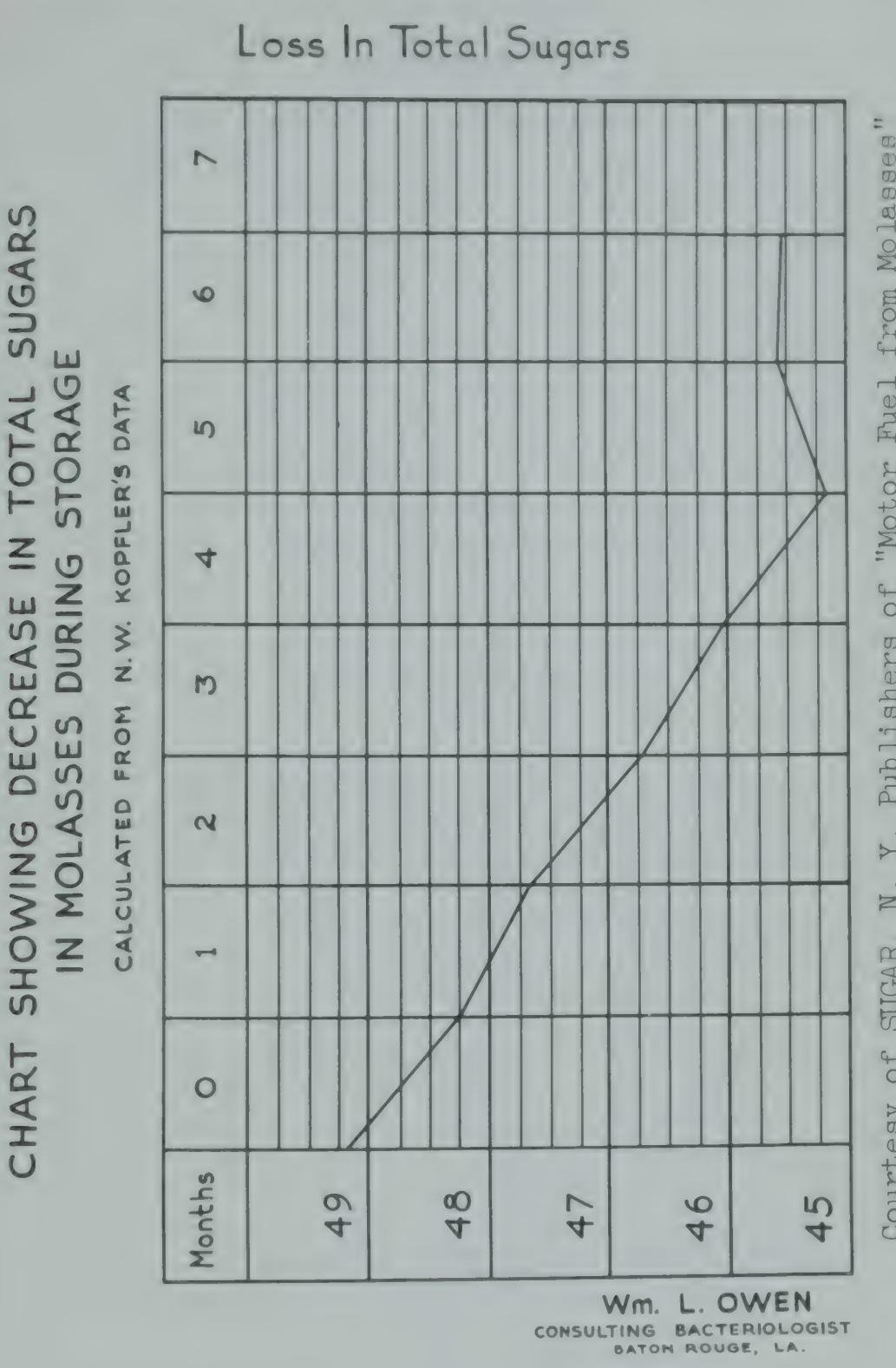
A seven months' period of observation of a deteriorating blackstrap is shown in the following table and chart from Geerlig's (12) citation from Kopfler's publication.

Analyses	Months							
	0	1	2	3	4	5	6	7
Brix	89.23	88.94	88.55	88.39	88.16	87.99	88.16	88.02
Clerget	37.75	37.27	36.96	36.22	35.76	35.37	35.11	34.98
Glucose	11.48	10.98	10.70	10.48	10.25	9.82	10.47	10.58
Total Sugar	49.23	48.25	47.66	46.70	46.01	45.19	45.58	45.56
pH	5.92	5.66	5.57	5.48	5.32	5.28	5.24	5.23
Color	18.02	24.47	29.78	32.39	36.21	38.27	35.25	35.49
	Loss in Total Sugars 3.67 Pct.							

(Chart on following page.)

That blackstraps of today can deteriorate with astonishing rapidity may be seen from the following data obtained in the course of an investigation of this phenomenon by the writer in recent years. (See data on pages 202, 203.)

Microbiological studies were made of these deteriorated samples, in order to determine whether the concentration of viable microorganisms was indicative or suggestive of their responsibility for this deterioration. These analyses included both mesophilic and thermophilic bacteria and also mold and yeast. The results are given in the following table. (See table on page 203.)


Loss In Total Sugars
CHART SHOWING DECREASE IN TOTAL SUGARS IN MOLASSES DURING STORAGE
CALCULATED FROM N.W. KOPFLER'S DATA
Months
0
1
2
3
4
5
6
7
49
48
47
46
45
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SHIPMENT OF BLACKSTRAPS

FACTORY NO. 1

Data	Brix	Total Sugars
Original Analysis	89.15	57.84
March 14, 1946	89.24	57.55
May 14, 1946	88.80	55.30
May 15, 1946	89.76	56.08
May 16, 1946	88.80	55.82
May 27, 1946	89.60	56.95
Aug. 22, 1946	87.44	53.47
Sept. 19, 1946	87.64	52.30
Oct. 26, 1946	87.74	52.32
Jan. 31, 1947	37.88	51.80

SHIPMENT OF BLACKSTRAPS

FACTORY NO. 2

Date	Brix	Sucrose	Glucose	Total Sugars
Original Analysis	88.29	37.49	22.47	59.96
Feb. 28, 1947	88.00	37.27	23.04	60.31
March 1, 1947	88.10	37.15	23.44	60.59
March 14, 1947	88.20	37.01	22.70	59.71
March 20, 1947	88.00	36.60	22.90	59.50
April 22, 1947	87.70	37.21	21.38	58.59
April 29, 1947	87.60	37.57	20.79	58.36
May 6, 1947	87.80	37.54	20.91	58.45
May 28, 1947	87.40	37.84	20.68	58.52
June 5, 1947	87.20	37.12	20.53	57.65
June 16, 1947	86.40	36.34	19.10	55.44
June 23, 1947	86.76	36.24	19.01	55.25
June 28, 1947	86.64	36.44	18.91	55.35

SHIPMENT OF BLACKSTRAPS

FACTORY NO. 3

Date	Brix	Sucrose	Glucose	Total Sugars
Original Analysis	88.25	36.20	22.49	58.73
March 11, 1947	87.80	36.44	22.34	58.78
March 20, 1947	88.20	36.79	22.38	59.17
March 20, 1947	87.60	36.83	22.07	58.90
April 11, 1947	88.00	36.99	21.54	58.53
April 18, 1947	87.50	37.09	21.61	58.70
April 29, 1947	88.00	37.94	20.71	58.65
June 14, 1947	86.40	37.34	19.42	56.76
June 18, 1947	87.14	36.79	18.87	55.66
June 19, 1947	87.23	36.01	19.11	55.12
June 26, 1947	86.56	36.50	18.87	55.37
July 2, 1947	86.88	36.40	18.63	55.03

	No. of Yeasts per gram		Mesophilic Bacteria		Thermophilic Aerobes		Thermophilic Bacteria		
Sample No.	Sucrose agar	W & S agar*	Aerobes	Anaerobes	Flat Sours	Total	Anaerobes -H2S	Anaerobes +H2S	Fungi
1	10	[illegible]	[illegible]						[illegible]
2	[illegible]	[illegible]	[illegible]						10
3	[illegible]	[illegible]	[illegible]						[illegible]
4	[illegible]	[illegible]	[illegible]						[illegible]
5	[illegible]	[illegible]	[illegible]						[illegible]
6	[illegible]	[illegible]	[illegible]						[illegible]
7	[illegible]	[illegible]	[illegible]						[illegible]
8	[illegible]	[illegible]	[illegible]						[illegible]
9	[illegible]	[illegible]	[illegible]						[illegible]
10	[illegible]	[illegible]	[illegible]						
11	[illegible]	[illegible]	[illegible]						[illegible]
12	[illegible]	[illegible]			[illegible]	[illegible]	--+---+-++--		
13	[illegible]	[illegible]			[illegible]	[illegible]	----------		[illegible]

* de Whalley and Scarr (Chemistry and Industry, Aug. 30, 1947)

It will be seen from the data in the above table that the numbers of viable microorganisms in these samples are insignificant and could not be regarded as a probable cause of the deterioration.

In a recent publication by Ozil (13) on a case of spontaneous carbonization of beet molasses stored in pits, he assigns to bacteria a significant role as contributory factors. However, no appraisals were made of the total numbers occurring in the deteriorated product and in this instance the molasses in question was made from badly deteriorated beets.

THE CHEMICAL NATURE OF THE SPONTANEOUS DETERIORATION OF BLACKSTRAP MOLASSES

We are indebted to Zerban (14) for his recent complete and comprehensive review of the literature on this phenomenon. It is interesting to note that as early as 1888, just three years after Durin (15) had published the first description of "frothing" fermentation of molasses, Classen (16) had opposed the fermentation theory of its origin on the grounds that microorganisms were unable to develop at such high levels of density of normal molasses. Lafar (17) regarded mold fungi as the causative agents in the phenomenon, while Herzfeld (18), as early as 1890, had shown that invert sugar solutions, when heated to a high temperature, darken and evolve carbon dioxide. Geerligs (19) regarded the action of lime upon glucic acid as the cause of this decomposition, while Gillet (20) claimed to have observed frothing in a massecuite which contained only traces of nitrogen. At the present time, there seems to be some unanimity of opinion to the effect that (1) the source of the evolved gas in the spontaneous fermentation is in the products formed from the action of invert sugars upon

amino and amide nitrogen in cane juices at high temperatures and is favored by high pH levels, (2) the susceptibility of blackstraps to this spontaneous fermentation or frothing increases with its density, that is it is more prevalent at very high levels of Brix, (3) over-liming and over-heating are highly important contributory causes, (4) the viscosities of the product greatly influence the susceptibility of blackstraps to deterioration and (5) that molasses susceptible to froth fermentation contains more non-fermentable sugars than those of normal behavior.

Zerban (21), in his review of this subject, presents the following steps in the synthesis of gas evolving complexes in molasses, viz: (1) the formation of N-glycosides, which are condensation products between reducing sugars and amino acids, and (2) the formation of melanoidins from the glycosides, as a result of further heating, which at high solid concentrations results in the eventual formation of dark brown to black amorphous substances, and the evolution of CO_2. In reviewing these various factors, we might well inquire the reasons for the apparently increasing susceptibility of modern cane blackstraps to this form of deterioration than the products of a former times. One of the most likely explanations is the increasing viscoscities of blackstraps, as a result of a more complete extraction of sucrose by modern methods of clarification and crystallization procedures. Shaw (22) has pointed out that Cuban blackstraps and probably those from all other countries have materially increased in viscosities during recent years and he offers, as an explanation of this condition, the following factors, Viz:

1. the higher milling extractions which recover more of the non-sugars in the cane,
2. the new varieties of cane which are now being milled in all countries.

To these explanations may possibly be added the occasional use of very old cane, which has been left standing over in the fields from one season to the next. High viscosities of molasses not only tend to aggravate the normal susceptibilities of these products to spontaneous fermentations, but the condition is accentuated by the enforced use of steam to permit of pumping the product from factory to storage tanks, and from the latter to tankers or cars. Hucker and Brooks (23) have demonstrated the relationship between density and foaming tendencies of molasses and the effect of a 25 percent dilution of a sample upon the volume of foam developed during storage at a temperature of 65° C. is shown in the following table appearing in their publication.

EFFECT OF ADDITION OF WATER ON VISIBLE FOAMING OF MOLASSES

	Control - no water added	25 percent water added
Time (1940)	Foam as per cent of original molasses volume	Foam as per cent of original molasses volume
June 10 - 3 P.M	0	0
June 11 - 8 A.M.	20	3
June 12 - 9 A.M.	67	3
June 13 - 8 A.M.	110	25
June 14 - 8 A.M.	112	20
June 15 - 4 P.M.	110	25
June 17	108	27
June 19	108	36

These authors found that the critical temperature of storage varied with different blackstraps, but as a rule it ranged from 40 to 45° C. It can easily be assumed that much of the blackstrap produced at the present time is delivered to storage tanks from pump lines at this level of temperature.

What is needed at the present time to insure the stability of blackstraps in storage is some adequate and reliable criteria to apply to these products, as they are produced, upon which to base predictions as to their probable stability in storage.

In an investigation of deteriorating samples of blackstraps, the writer endeavored to develop criteria that would serve this purpose and it would seem that some of these may have considerable significance in this connection. At the outset, it appeared evident that no known chemical method can detect these latent addictions to deteriorative changes in storage. Hucker and Brooks (24) attempted to correlate excessive foaming of blackstraps with their calcium content, but found that there was no consistent agreement between these values. The results of their investigations of this phase of the problem is shown in the following table from their publication. (See table on page 208.)

These authors concluded that the greatest predisposing factor in the phenomenon of frothing molasses is the temperature at which the product is stored.

The first question to be answered in the determination of the susceptibility of blackstraps to deterioration is whether or not there is any definite correlation between their tendency to evolve gas in frothy fermentation and their actual

CALCIUM OXIDE CONTENT OF FINAL MOLASSES
(PLANTS A AND C)

Sample		Calcium Oxide in final molasses			
No.	Source	1939		1940	
		Plant A - %	Plant C - %	Plant A - %	Plant C - %
1	First week of grind	1.80	....	1.80	1.44
2	Second " " "	2.02	1.84	1.50	1.30
3	Third " " "	2.04	2.22	1.54	1.78
4	Fourth " " "	2.46	2.10	1.68	1.94
5	Fifth " " "	2.32	2.18	1.86	1.98

Plant A - Molasses High Foaming Qualities in 1939

Plant C - Low Foaming Tendencies

deterioration in storage. It is well known, of course, that actual deterioration involving loss of sugars is accompanied by gas evolution, but it is also true that the latter cannot always be taken as an indication of the destruction of sugars. On the contrary, as has previously been noted, there is some evidence that "frothing massecuites" are less viscous and "purge" better in the centrifugals and give higher yields of crystals than those that do not. Practical methods have long been in use by canners of molasses to appraise the addiction of low-grade molasses to this spontaneous decomposition and while the procedures are by no means infallible, they do afford some measure of protection against losses from "swells and springers" in canned molasses. Practical experience has taught the canners of these products that in order to render them stable for canning, they must first be diluted and reboiled, before processing in the

Canning room. The presumption is that in reboiling, the decomposable bodies that evolve gas on storage are broken down and the product can then be safely canned if the temperature at which it is processed is kept within safe limits. But even with these precautions, some cooking grades of blackstraps cannot be prevented from undergoing further decomposition and causing swells and springers in storage, hence the product to be canned must be carefully selected by some preliminary test. The tests used may vary in simplicity from the mere exposure of the product in test tube to a temperature of boiling water, or it may consist in heating the sample to 105° C. in an oil bath and reading off the millimeters of mercury pressure developed in the sealed tube containing it. The readings are made, of course, after the contents of the tube have cooled to room temperature. The canner does not, as a rule, keep any data on the loss of solids resulting from the reboiling process, so the losses in this connection cannot be appraised and as canned molasses is not priced on a sugar basis there might be considerable actual deterioration in these values which would escape detection under the system of control operative in such plants.

In a recent investigation of the causes of the rapid deterioration of several lots of Cuban blackstraps, the writer attempted to correlate the rate of "frothing" or gas evolution of these samples and their loss of sugars in storage at high temperatures. In this investigation, the gas evolving tendencies of the samples were determined (1) by heating the samples to 105° C. and determining the cm. of mercury pressure that developed as a result. (2) incubating the samples at 55° C. in sealed bottles equipped with tubes filled with mercury and (3) exposing the products to a temperature of 55° C. in sealed cans and observing the

time required for "swells" to develop and correlating this factor with the actual chemical analyses of the products after this decomposition had taken place. In the first of these procedures the method for the test was as follows:

> 100 grams of the samples were introduced into bottles of the same type as previously used and the samples were then placed in a water bath and their temperatures brought up to 67° C. They were then placed in a glycerin bath, the temperature of which was on the same level, namely 67° C. The sample bottles were stoppered with rubber stoppers, with a connecting glass tubing provided with a glass stopcock. The temperature of the glycerin was then brought up slowly to 105° C., with the connecting tubing connected to a mercury manometer, and when the temperature of the sample reached 105° C. the glass stopcock was opened and the mercury pressure read on the tubes as mercury pressure. The glycerin bath was then cooled back to its original temperature of 67° C. and the final mercury pressure recorded at the point of equilibrium with the original pressure within the container.

In the second test, the following procedure was followed:

> 100 gram portions of each of the samples were weighed into 250 C.C. wide-mouth bottles. The bottles were stoppered with tightly fitting rubber stoppers, through which mercury pressure tubes were inserted. These tubes were so designed that a constant level of approximately 5 ccm. of

mercury was maintained, when the gas pressure within the bottle was at zero. With the increase in gas pressure, the pressure could be determined by the rise of mercury in the outer arm of these tubes. The samples were placed in a 55° C. incubator and when these had attained that temperature, the pressure tubes and stoppers were fitted to each bottle.

In the third test, the samples were placed in 2.5 pound friction-top cans and placed in a 55° C. incubator for twenty-four hours and at the end of this period, when the contents of the cans had reached that temperature, the tops were inserted and tightly sealed. Observations for "swells" and "springers" were made each day and those samples showing evidences of gas pressures were removed and their contents analyzed and compared with the Controls, which were kept at room temperatures. The results of these combined tests are shown in the following table: (See table on page 212.)

It will be seen from the following table that there is no correlation whatever, between the tendencies of the samples to evolve gas at high temperatures and their tendency to deteriorate under these conditions. For example, sample No. 11, which lost almost 4.0 per cent of total sugars, required longer to produce "swells" in cans and did not show the highest gas pressure at a temperature of 105° C., nor did it develop gas more rapidly at a temperature of 55° C. There is one definite correlation, however, that we have found to exist between the chemical composition of these samples as determined by analysis and their tendency to deteriorate at high temperature storage. We have found that those samples having the highest total sugar values, that is at levels of 59-60 percent, are the ones that are most susceptible to actual

						Change in Composition at 55° C. Storage in Cans					Fermentation Efficiency	
Sample No.	Area of Storage Tank	Pressure- Mercury CM. 105° C.	55° C.		Days Required to Show	Pressure	Days Required to Show	Loss in Sucrose	Loss in Reducing Sugars	Loss in Total Sugars	A at Room Temperature	B at 55° C Puffed
1	----	7.0	+		4	----	----	----	----	----	----	----
2	----	7.0	+	4.5	24	Not tested - insufficent sample +	14					
3	Top	7.0	+		4	+	8					
4	Middle	10.0	+	2.0	4	+	12					
5	Bottom	10.5	+		9	+ Puffed	1 week	0.83	+ 0.22	0.61	91.7	85.03
6	Top	9.0	+	4.0	4							
7	Middle	15.5	+		4							
8	Bottom	9.0			4							
9	Top	7.0	+		4							
10	Middle	10.0	+ +		3.5	+	14	-.47	+ .20	0.27		
11	Bottom	10.5	+		9	+ Puffed	11	0.91	- 2.99	3.90	85.46	88.06
12	Top	7.5	+ +		4							
13		15.0	+ +		4							
14	Control	5.5	+	5.5	3 weeks	---	3 weeks					

deterioration in storage and the samples with total sugars of 54-55 per cent very rarely deteriorate further, when stored at high temperatures. For example, and as an illustration, we have the following:

Sample Number	Total Sugar	Loss in Sugars at 55° C.	Time of Storage
11	59.43	6.90	11 days
3	59.85	0.61	7 "
4	58.01	0.55	4 "
10	57.64	0.27	14 "
2	54.74	0.0	14 "

The samples having a total sugar content of approximately 59 per cent total sugars deteriorated very rapidly, while the others at lower levels of sugar withstood a two weeks incubation at this temperature with scarcely any changes whatever. The most probable explanation of this difference is that the molasses samples at this lower level of total sugar value had probably attained stability from the deterioration that had previously taken place, for the original analyses of these products previously presented, indicate a sugar value of from 58 to 60 percent total sugars.

THE UNFERMENTABLE SUGARS IN DETERIORATED BLACKSTRAPS

The close relationship between the unfermentable reducing sugars in and the "frothing" tendencies of blackstraps has been pointed out by Guerrero (25), who found in his investigations

that "frothing" molasses contain more unfermentable sugars than do normal ones. As an illustration of this relationship is shown our fermentation tests of a blackstrap before and after high temperature storage, during which period it had evolved considerable amounts of gas. The original samples contained 59.85 per cent total sugars and after one week's storage at 55° C., the total sugar value fell to 59.24 and sufficient gas pressure had been developed to puff the can containing it. Fermentation tests on the samples before and after storage were as follows:

A - At room temperature - Fermentation efficiency - 91.7

B - At 55° C. for one week - Fermentation efficiency - 85.03

The efficiency in the latter case was determined on the basis of the actual sugar content, as determined after storage.

Another example of the fermentability of a deteriorated blackstrap which underwent a drastic loss of sugar during high temperature storage is given below.

A - Stored at room temperature - Total Sugars - 59.43
Fermentation efficiency - 88.06

B - Stored at 55° C. for 11 days - Total Sugars - 55.43
Fermentation efficiency - 85.46

Efficiency calculated on actual sugars as determined by analysis after storage.

In these examples we have an illustration of low efficiencies of fermentation on blackstraps, which, on the one hand, had undergone "frothy" fermentation without material loss in sugars, and on the other had suffered actual sugar deterioration in storage. These two illustrations explain many of the difficulties experienced by distillers of blackstrap in obtaining satisfactory levels of fermentation efficiencies from these products after they have been in storage for long periods of time.

We may conclude from these tests, and many others of this kind, that irrespective of the sugar values of old blackstraps, which have undergone "frothy" fermentation, the sugars, or rather the reducing bodies regarded as sugar, include a relatively higher percentage of unfermentable reducing substances than is true of fresh blackstraps.

It is interesting to observe, in the illustration given by Browne (26) of the deterioration of a blackstrap over a period of eleven years, that the sugar losses consisted entirely of losses in sucrose, which amounted to 18.60 per cent, with a gain in invert sugar, which gave a net loss during that period of 15.20 per cent. Kopfler's (27) data also shows a loss of total sugars in seven months of 3.67, of which the loss in sucrose constitutes, by far, the greater part. On the other hand, the cases which we have observed of rapid deterioration in a few days at high temperatures, or over a period of months under factory conditions, the losses have been largely at the expense of glucose, rather than of sucrose. As an illustration, we give the following data from actual records previously cited, Viz.:

	Total Loss in Sugars	Sucrose Loss
After 4 months storage	4.96	0.83
After 4 " "	3.75	1.04
After 9 " "	3.86	0.66

Similarly in our tests of the deterioration of blackstraps at high temperature storage for 11 days, we have as an illustration the following:

Total Loss of Sugar.....3.90

Sucrose Loss.....0.91

Neuville's (28) data on the deterioration of blackstraps in tank and pit storage also indicates losses largely at the expense of sucrose and so does the publication by Geerligs (29), in which Kopfler's data is cited. It might appear from the foregoing data that slow deterioration of these products is largely a loss of sucrose as such or the decomposition of the invert sugar formed from it, if inversion precedes this loss. On the other hand, the rapid decomposition or deterioration of blackstraps is largely at the expense of the invert sugar already present. A fair interpretation of the latter instance is the decomposition of reducing substances that are in fact not sugars at all but more likely N-glycosides, which have reducing action upon Fehling solution and are included in the assay of invert sugar.

Unfortunately, fermentation tests of molasses do not always reveal the presence of these glycosides, owing to the fact that in acidified mashes they are reconverted to their original components, hence satisfactory fermentation efficiencies may be obtained on blackstraps which

contain relatively large amounts of them. On prolonged storage, these glycosides may undergo further decomposition and be converted into melanoidins, which undergo further decomposition with the evolution of gas and the formation of unfermentable reducing substances.

CONCLUSIONS

1. Contrary to previous concepts on the subject, modern blackstraps can no longer be regarded as even fairly impervious or resistant to deterioration in storage and under certain conditions may deteriorate very rapidly.

2. The evolution of gas or the tendency of these products to under "frothy" fermentation cannot be correlated with their tendency to lose sugars in storage.

3. The deterioration of blackstraps is definitely not due to the action of microorganisms, but is entirely autogenous in origin.

4. Irrespective of whether the spontaneous fermentation is or is not associated with actual losses in sugar values, the fermentation efficiency is always adversely affected by it.

5. There appears to be two main types of blackstrap deterioration, namely those which proceed at a normal rate during a period of years and in which the losses are principally from the destruction of sucrose, and those which are far more rapid and in which the losses are almost entirely at the expense of invert sugars.

6. It is the writer's opinion that the increasing viscosities of present day blackstraps and their high total sugar content make them increasingly vulnerable to deterioration in storage.

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